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Systemic infection of petunia by mechanical inoculation with tomato golden mosaic virus

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The geminiviruses are a group of plant viruses characterized by particles with twin icosahedral morphology, genomes of covalently closed circular single-stranded DNA molecules, 2.5 to 3.0 kb in size, and capsids composed of single polypeptide species, M_r = 28 000 to 30 000 (Stanley, 1985). Geminiviruses which in nature are transmitted by leafhopper vectors, e.g. maize streak virus, beet curly top virus, have undivided genomes (Grimsley et al., 1987; Stanley et al., 1986), whereas the whitefly-transmitted geminiviruses, e.g. tomato golden mosaic virus, bean golden mosaic virus and cassava latent virus, have bipartite genomes (Hamilton et al., 1983; Morinaga et al., 1983; Stanley, 1983).

Tomato golden mosaic virus (TGMV) possesses a host range within the *Solanaceae* and several different *Nicotiana* L. species can be infected mechanically with the virus with high efficiency (Buck and Coutts, 1985). Mechanical transmission of the virus from tomato (*Lycopersicon esculentum* L.) to tomato has been achieved with difficulty whereas mechanical infection of *Solanum pennelli* and derivatives of crosses between *L. esculentum* and *S. pennelli* is easy (Costa, 1976). Typical symptoms of infection on young plants of susceptible species and cultivars consist of stunting with severe deformation of young leaves and shoots, accompanied by a bright yellow mosaic (Costa, 1976; Hamilton et al., 1981). Older plants often 'recover' from infection and produce apparently healthy leaves which later develop sporadic symptoms (Buck and Coutts, 1985).

Rogers et al., (1986) were unable to infect petunia with TGMV by mechanical inoculation. However when tandem direct repeats of the A component DNA were introduced into petunia and integrated into the chromosomal DNA using a Ti plasmid vector, unit length viral DNA molecules were produced, replicated and encapsidated into virus particles, although no symptoms were observed. No unit length viral DNA molecules were observed in transgenic plants containing direct repeats of B component DNA, but in plants transgenic for both components (A × B hybrids) production and replication of unit length A and B component DNA circles were observed and the plants showed symptoms of infection. Although difficulties in demonstrating the infectivity of crude virus preparations were initially experienced (Rogers et al., 1986) subsequently, it was found that DNAse treated virions isolated from these plants were

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infectious when inoculated onto healthy *N. benthamiana* (Sunter et al., 1987). On the basis of these results and the fact that both TGMV DNA components are required to infect *N. benthamiana* (Hamilton et al., 1983) it was suggested that DNA A encodes all viral functions necessary for replication and that DNA B determines virus cell-to-cell transport and development of symptoms (Rogers et al., 1986; Sunter et al., 1987). The results also show that petunia cells can support the replication of TGMV DNA. However because of the variability of symptoms in different transgenic (A × B hybrid) plants, development of symptoms predominantly in older, rather than younger, leaves, attainment of only low levels of infectivity and the failure to transmit the virus to petunia by mechanical inoculation it was suggested that symptom development depended on localised cell-to-cell spread of the virus and that TGMV may not be capable of inducing a systemic infection in petunia. If this is so, the virus replication and symptom development in petunia plants transgenic for both TGMV DNA components would be an example of extending the host range of a virus *via* plant transformation with a Ti plasmid vector. Here we show that in fact TGMV can be transmitted to petunia by mechanical inoculation and that the plants become infected systemically with the virus.

N. benthamiana and Petunia hybrida F_1 'Resisto Mixed' (Suttons seeds, United Kingdom) plants were cultivated as previously (Adejare and Coutts, 1982) until they reached the 6 to 8 leaf stage some 4 to 6 weeks after potting out. All plants were watered daily and fed weekly with Phostrogen (Phostrogen Ltd., United Kingdom) fertilizer. Petunia plants were dusted with 500 mesh carborundum and inoculated with TGMV prepared from infected *N. benthamiana* by a modification of the method of Stein et al., (1983). Systemically infected leaves were mixed with 2 ml/g CEM buffer (0.1 M trisodium citrate, 0.75% (w/v) sodium sulphite, 5 mM disodium EDTA, 1% (v/v) 2-mercaptoethanol, pH 7.0) and homogenised. The homogenate was made 2.5% by volume in Triton X-100, stirred at 4 °C overnight and filtered through muslin. The filtrate was cleared by centrifugation at 10 000 *g* for 15 min and the supernatant was centrifuged at 40 000 rpm for 2 h at 4 °C in a Beckman type 65 fixed-angle rotor to

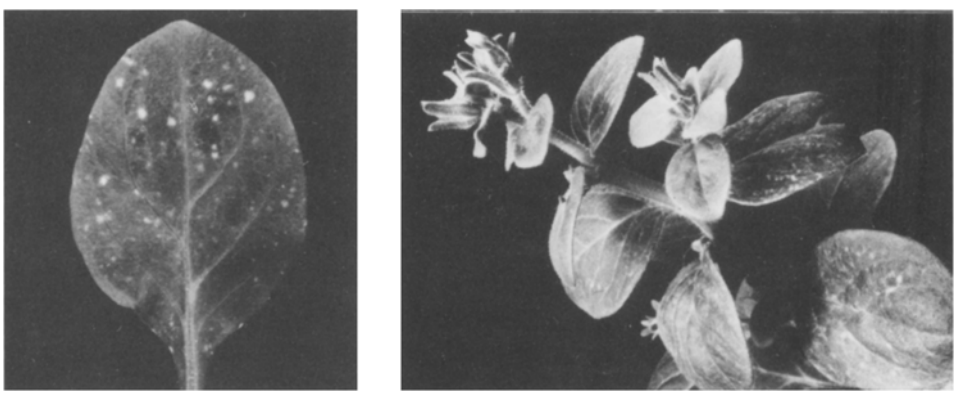

Fig. 1. Symptoms of TGMV infection of *Petunia hybrida*. Left: Close-up view of systemically infected petunia leaf showing the characteristic circular, yellow lesions. Right: Systemic infection of whole plants, two weeks after infection.

pellet the virus. Each pellet was resuspended in 1 ml sterile distilled water for immediate use as an inoculum.

Two weeks after inoculation circular yellow lesions were clearly visible on the inoculated leaves similar in form to those described previously on A × B crossed transgenic plants (Rogers et al., 1986). Similar lesions gradually appeared on other, younger leaves on the plants over a period of 4 to 6 weeks (Fig. 1). No severe distortion of the leaves was observed.

Six weeks after inoculation, virus was prepared from systemically infected leaves, as described above for TGMV-infected *N. benthamiana*. DNA was extracted and analysed by agarose gel electrophoresis and Southern blotting, alongside similarly prepared virus from systemically-infected *N. benthamiana*, as described by Hamilton et al. (1982). Blots were hybridised to a mixture of nick-translated plasmids pBH401 (TGMV DNA A) and pBH604 (TGMV DNA B) (Bisaro et al., 1982). A band of viral DNA which comigrates with authentic TGMV ssDNA was detected from the systemically infected petunia leaves (Fig. 2). When virus prepared from systemically infected petunia leaves was inoculated to healthy *N. benthamiana* plants, symptoms typical of TGMV infection were produced after 2 weeks.

In this study we have demonstrated that TGMV can be transmitted to Petunia hybrida by mechanical inoculation and establish a systemic infection and that the replication of TGMV DNA and development of symptoms in petunia transgenic for both TGMV DNA components is not an example of extending the host range of the virus by genetic engineering.

The reason for the inability of Rogers et al., (1986) to transmit TGMV to petunia by mechanical inoculation could be that the hybrid used by these investigators (Mitchell) is more resistant than those used in the present study, although we have found several different F_1 hybrid plants to be equally susceptible. On the other hand it is not surprising that symptom development in the transgenic plants is different from that in mechanically inoculated untransformed plants. In the transgenic plants every

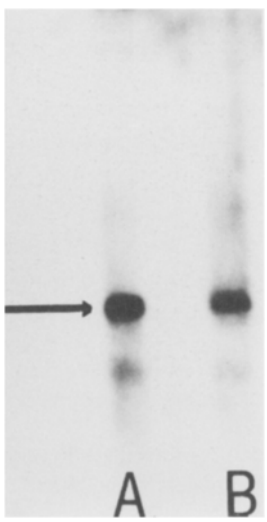

Fig. 2. Autoradiograph of Southern blotted gel containing nucleic acid extracts from systemically infected *N. benthamiana* tissue (track A) and systemically infected *P. hybrida* (track B). The position of virion single-stranded DNA is arrowed. The transfer was onto a nitrocellulose membrane.

cell carries integrated copies of the TGMV genome. Since replication of the B component requires the A component, but not *vice versa*, there may be a significant proportion of cells in which only DNA A is replicating and producing TGMV coat protein (Sunter et al., 1987). Even cells which contain only the integrated copies could produce some coat protein by expression from the nuclear genome. Such cells producing coat protein may be relatively resistant to superinfection by virus spreading from cells in which both A and B components are replicating and hence a systemic infection may be more difficult to establish. This could be similar to the protective effect seen in transgenic plants expressing the coat protein of tobacco mosaic virus (Abel et al., 1986) and alfalfa mosaic virus (Tumer et al., 1987) or in the case of cells replicating only DNA A particles to plant virus cross-protection in general in which coat protein is only one of several protective mechanisms (Fulton, 1986).

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Samenvatting

Systemische infectie van petunia na mechanische inoculatie met het 'tomato golden mosaic virus'

Aangetoond werd dat *Petunia hybrida* systemisch kan worden geïnfecteerd met het 'tomato golden mosaic virus' (TGMV), een virus dat behoort tot de groep van de geminivirussen. Mechanische inoculatie van petuniaplanten met TGMV gaf in de systemisch geïnfecteerde bladeren symptomen, die eerder in een aantal andere Solanaceae waren waargenomen. Daar in eerdere proeven petunia niet met TGMV kon worden geïnfecteerd en DNA-replicatie en symptoomontwikkeling wel optrad in, voor de beide genomen van het virus, transgene planten, werd gesuggereerd dat het hier een geval betrof van uitbreiding van de waardplantenreeks.

De hier gepresenteerde resultaten kunnen echter tot andere conclusies leiden. Het is namelijk mogelijk, dat bepaalde F_1-hybriden van petunia resistenter zijn tegen het virus. Verschillen in de symptoomontwikkeling zijn echter ook niet uit te sluiten en zouden veroorzaakt kunnen worden door premunitie als gevolg van de aanwezigheid van het manteleiwit in opnieuw geïnfecteerde cellen.

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