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Research Notes

Limited Replication of Tomato Golden Mosaic Virus DNA in Explants of Nonhost Species

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Agroinoculation of the cloned genome components of tomato golden mosaic virus (TGMV) produces systemic infection of *Nicotiana benthamiana*, but not tomato, sugar beet, or *Arabidopsis thaliana*. However, upon agroinoculation of explant tissue, limited TGMV DNA synthesis was observed in all three nonhost species, indicating that the host range restriction is not due to an absolute inhibition of replication.

Additional keywords: geminivirus, host range, subliminal infections.

Tomato golden mosaic virus (TGMV) is a whitefly-transmitted member of the geminivirus group (for reviews see Lazarowitz 1987; Bisaro *et al.* 1990). The encapsidated genome of TGMV consists of two circular, single-stranded DNA species, designated A and B, which replicate via double-stranded intermediates (Hamilton *et al.* 1982). Recent studies suggest that replication of the 2.6-kb genome components is accomplished by a rolling-circle mechanism (Saunders *et al.* 1991; Stenger *et al.* 1991). Sequence analysis of TGMV (Hamilton *et al.* 1984) has indicated that DNA A contains four open reading frames (ORFs) that encode all viral proteins required for DNA replication and encapsidation, whereas DNA B contains two functional ORFs encoding viral proteins necessary for systemic movement (Rogers *et al.* 1986; Sunter *et al.* 1987, 1990).

Early reports concerning a whitefly-transmitted viral agent inducing a golden mosaic of tomato (*Lycopersicon esculentum* Mill.) in Brazil have been reviewed (Costa 1976; Buck and Coutts 1985). Characterization of the virus by Hamilton *et al.* (1981) confirmed TGMV as a member of the geminivirus group, with a known host range restricted to species of the Solanaceae (Buck and Coutts 1985). The genome components of TGMV were cloned from extracts of infected *Nicotiana benthamiana* Domin. (Bisaro *et al.* 1982), and infectivity of the cloned genome components was demonstrated by mechanical inoculation on *N. benthamiana* (Hamilton *et al.* 1983). However, infection of tomato with cloned TGMV DNA inoculum has not been reported. In this communication, we describe host range experiments designed to test the ability of cloned TGMV DNA to infect the original host of the virus (tomato), and of two species outside of the Solanaceae; sugar beet (*Beta vulgaris* L.) and *Arabidopsis thaliana* Heyn. The results of studies that examined the ability of cloned TGMV DNA to replicate in leaf disc or root piece explants are also reported.

Infectivity assays of whole plants were performed by *Agrobacterium*-mediated inoculation (agroinoculation; Grimsley *et al.* 1987; Grimsley and Bisaro 1987). *A. tumefaciens* strain GV3111 harboring binary Ti plasmids containing tandemly repeated copies of TGMV DNA A (pMON337) or TGMV DNA B (pMON393) have been described previously (Elmer *et al.* 1988b). In plant cells, unit-length viral genomes are released from tandem viral genome repeats by a replicative mechanism or by homologous recombination (Elmer *et al.* 1988b; Stenger *et al.* 1991). Bacteria containing plasmids pMON337 or pMON393 were coinoculated onto cut stems of *N. benthamiana*. Agroinoculation of TGMV DNA to tomato (cultivars SGCR26, New Yorker, and Cherry), sugar beet (cultivar SS2), and *A. thaliana* (land race No-O) was performed by needle puncture (Briddon *et al.* 1989), a procedure previously demonstrated to be efficient in delivering the monopartite beet curly top geminivirus (BCTV) to these species (Stenger *et al.* 1992; Davis 1992). A binary Ti plasmid (pMLOGAN) containing a tandemly repeated copy of the BCTV-Logan strain genome (Stenger *et al.* 1991) was agroinoculated to whole plants as a positive control. Three to four weeks postinoculation, upper leaves were extracted for total DNA (Stenger *et al.* 1992), and the infection status of each individual plant was determined by Southern or dot blot hybridization. The results of whole plant infectivity assays indicated that only *N. benthamiana* was infected following agroinoculation with TGMV DNA (Table 1, Fig. 1). Systemic symptoms of TGMV infection were typical and consisted of a bright yellow mosaic, leaf distortion, and stunting. No symptoms were observed on tomato, sugar beet, or *A. thaliana* plants agroinoculated with TGMV DNA, nor was TGMV DNA detected in leaf samples from these species (Fig. 1). In contrast, systemic infection of all four species was observed with BCTV (Table 1), indicating that the agroinoculation procedure

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employed delivered the geminivirus DNA inoculum efficiently to the plant species tested.

The ability of cloned TGMV DNA to replicate in host and nonhost species was examined by agroinoculation of leaf discs from *N. benthamiana*, tomato and sugar beet, or root pieces from *A. thaliana*. The leaf disk assay for replication of TGMV DNA in *N. benthamiana* has been described (Elmer *et al.* 1988a). Tomato (cultivar SGCR26) and sugar beet (cultivar SS2) discs were prepared and agroinoculated by the same procedure. Root piece explants of *A. thaliana* were used in preference to leaf disks because discs from this species are fragile and easily damaged by surface sterilization procedures. *A. thaliana* (land race No-O) root pieces were obtained from 2-wk-old sterile seedlings grown in liquid B5 medium (Gibco), pH 5.7. Root pieces (5–10 mm) were excised and placed in liquid callus inducing medium (B5 medium, pH 5.7, with 0.5 mg/ml 2-[*N*-morpholino]ethanesulfonic acid (MES), 1 μg/ml 2,4-dichlorophenoxyacetic acid, and 0.05 μg/ml kinetin) and incubated for 3 days (20° C, 12-hr daily photoperiod) on a shaker operating at 50 rpm. Root pieces were then co-cultivated in liquid callus inducing medium with an equal mixture of *A. tumefaciens* cultures containing pMON337 and pMON393, or pMLOGAN alone, and incubated (20° C, 12-hr photoperiod, 50 rpm) for two additional days. Root pieces were washed with liquid B5 medium, pH 5.7, and incubated (20° C, 12-hr photoperiod) on solid medium (B5 medium, pH 5.7, 0.5 mg/ml of MES, 0.15 μg/ml of indole-3-acetic acid, 5 μg/ml of N^6-[2-isopentenyl] adenine, 50 μg/ml of kanamycin, 500 μg/ml of carbenicillin, and 0.2% Gelrite [Schweizerhall]). After a 5-day incubation period, explants were surface sterilized and extracted for total DNA.

Table 1. Host range of cloned tomato golden mosaic virus (TGMV) and beet curly top virus (BCTV) DNA delivered to whole plants by agro-inoculation

Plant species	Infectivity[a]		
	TGMV Exp. 1	TGMV Exp. 2	BCTV
Nicotiana benthamiana	8/8	4/4	8/8
Tomato			
SGCR26	0/18	0/8	7/8
New Yorker	ND	0/8	ND
Cherry	ND	0/8	ND
Sugar beet	0/8	0/8	8/8
Arabidopsis thaliana	0/8	0/8	8/8

[a]Number of plants systemically infected/number of plants inoculated, ND = not determined.

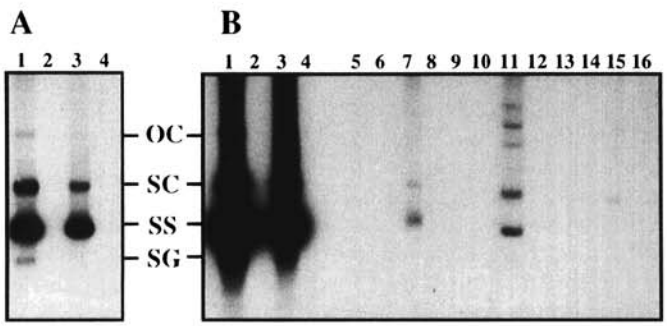

Fig. 1. Southern blot analysis of total DNA (1 μg of *Nicotiana benthamiana* DNA, 2 μg of tomato, *Arabidopsis thaliana*, and sugar beet DNA) extracted from whole plants or explants agroinoculated with cloned tomato golden mosaic virus (TGMV)DNAs A and B. Representative samples denoted by numbers are: 1, TGMV inoculated *N. benthamiana*; 2, uninoculated *N. benthamiana*; 3, TGMV inoculated *N. benthamiana* leaf discs; 4, pMON521 (Ti plasmid vector without geminivirus sequences) mock-inoculated *N. benthamiana* leaf discs; 5, TGMV inoculated tomato; 6, uninoculated tomato; 7, TGMV inoculated tomato leaf discs; 8, pMON521 mock-inoculated tomato leaf discs; 9, TGMV inoculated *A. thaliana*; 10, uninoculated *A. thaliana*; 11, TGMV inoculated *A. thaliana* root pieces; 12, pMON521 mock-inoculated *A. thaliana* root pieces; 13, TGMV inoculated sugar beet; 14, uninoculated sugar beet; 15, TGMV inoculated sugar beet leaf discs; and 16, pMON521 mock-inoculated sugar beet leaf discs. Hybridization was conducted using a TGMV DNA A-specific riboprobe complementary to virion-sense DNA. High molecular weight DNA forms in lane 11 are multimeric forms of viral DNA and residual Ti plasmid inoculum. **A,** A short autoradiograph exposure (8 hr) of lanes 1–4; **B,** a long autoradiograph exposure (6 days) of lanes 1–16. Open circular (OC), supercoiled (SC), single-stranded (SS), and subgenomic (SG) forms of replicating TGMV DNA are indicated.

Southern hybridization analysis of total DNA samples from explants revealed that TGMV-agroinoculated *N. benthamiana* leaf discs contained high levels of both single-stranded and double-stranded viral DNA forms, as expected (Fig. 1). Interestingly, low levels of viral DNA were detected in extracts of all three nonhost species when explants were agroinoculated with TGMV. No viral DNA was detected in extracts from explants agroinoculated with a control binary Ti plasmid (pMON521) lacking TGMV sequences. Some reduction in the amount of viral DNA might be expected in explants from species that do not support virus movement. However, viral DNA levels in nonhost explants were much less than those observed in *N. benthamiana* explants agroinoculated with movement defective TGMV DNA B mutants, which typically show

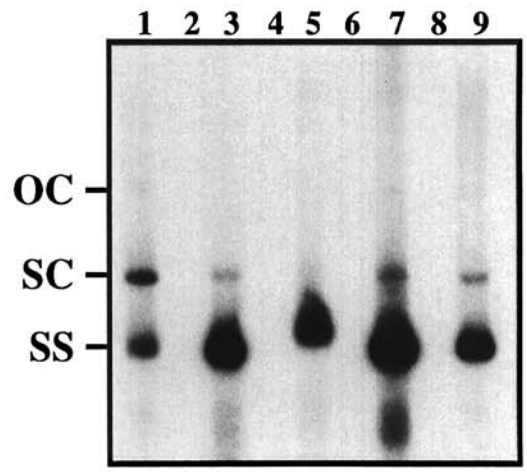

Fig 2. Southern blot analysis of total DNA (1 μg of *Nicotiana benthamiana* DNA, 2 μg of tomato, *Arabidopsis thaliana*, and sugar beet DNA) extracted from whole plants or explants agroinoculated with cloned beet curly top geminivirus (BCTV) DNA. Samples denoted by numbers are: 1, BCTV inoculated sugar beet; 2, pMON521 mock-inoculated *N. benthamiana* leaf discs; 3, BCTV inoculated *N. benthamiana* leaf discs; 4, pMON521 mock-inoculated tomato leaf discs; 5, BCTV inoculated tomato leaf discs; 6, pMON521 mock-inoculated *A. thaliana* root pieces; 7, BCTV inoculated *A. thaliana* root pieces; 8, pMON521 mock-inoculated sugar beet leaf discs; and 9, BCTV inoculated sugar beet leaf discs. Hybridization was conducted using a BCTV-specific riboprobe complementary to virion-sense DNA. Duration of autoradiography was 48 hr. Open circular (OC), supercoiled (SC), and single-stranded (SS) forms of replicating BCTV DNA are indicated.

only a three- to fourfold reduction in DNA levels relative to wild-type virus (unpublished observations). Therefore, the greatly reduced levels of TGMV DNA detected in explants of nonhost species was likely due not only to the absence of systemic movement, but also to a reduction in viral DNA replication. The presence of BCTV viral DNA forms, in substantial amounts, in extracts from agro-inoculated explants of all four species (Fig. 2) indicated that the explant systems were able to support efficient replication of a virus known to systemically infect the species tested.

Our results suggest that TGMV produces a localized, subliminal infection in the three nonhost species tested. Thus, host range restriction probably results from the inability of TGMV to move systemically from the initial point of infection. Impaired systemic movement may be the result of incompatible viral movement genes. Alternatively, it is possible that TGMV replication levels in nonhost species are too low to provide sufficient amounts of movement proteins and/or viral DNA to facilitate movement. We regard the latter possibility as less probable since a TGMV AL3 ORF mutant retains infectivity and systemic movement in *N. benthamiana* despite a 50-fold reduction in DNA synthesis (Elmer *et al.* 1988a; Sunter *et al.* 1990). Studies with a restricted host-range isolate of a related geminivirus, squash leaf curl virus, suggest that both replication and movement deficiencies may contribute to host range determination (Lazarowitz 1991).

Replication of TGMV DNA at low levels in cells of nonhost species has several interesting consequences. First, replication in explants of *A. thaliana* indicates that this species, a tractable genetic system, may be used in transient viral replication and gene expression studies. In addition, there are evolutionary implications. Localized infections in nonhost species may facilitate the divergence of virus populations by allowing variants better adapted to the plant to arise. Subliminal infections may also provide an opportunity for related viruses with distinct host ranges to recombine and exchange genetic material. Both of these mechanisms may have contributed to the present diversity of geminiviruses.

The inability of cloned TGMV DNA to initiate a systemic infection in tomato, the original host of the virus, precludes authentication of the cloned virus genome as the causal agent of tomato golden mosaic disease. As we have not been able to test tomato cultivars known to be susceptible to the original virus isolate, the lack of systemic infection of the three cultivars used in our study may reflect differential resistance. However, this possibility seems unlikely because resistance to geminiviruses known to infect *L. esculentum* has not been reported. We believe it is more likely that this isolate of TGMV lost the ability to infect tomato during passage in *Nicotiana* spp. prior to cloning.

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