Absence of Lethal Stem Necrosis in Select *Lycopersicon* spp. Infected by Cucumber Mosaic Virus Strain D and Its Necrogenic Satellite CARNA 5

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ABSTRACT

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Fifty-two *Lycopersicon* spp. accessions were inoculated with cucumber mosaic virus (CMV) strain D containing the satellite CARNA 5 (= CMV-associated RNA 5) that induces tomato necrosis in *L. esculentum* 'Rutgers'. Many exotic species showed only mosaics or chlorosis in spite of large accumulation of CMV and CARNA 5. CMV was purified from several infected accessions for further analysis of its CARNA 5. All CARNA 5s isolated from infected accessions migrated to the same position under semidenaturing polyacrylamide gel electrophoresis as necrogenic CARNA 5 from CMV-D used for inoculation. Partial nucleotide sequencing of several CARNA 5s showed them to be identical to D-CARNA 5. The observation that many *Lycopersicon* spp. support the replication of CMV and CARNA 5 efficiently but do not respond necrotically suggests that these accessions may have gene(s) that block the necrotic response or may lack gene(s) that induce necrosis.

Cucumber mosaic virus (CMV) is composed of four single-stranded RNAs (numbered 1–4 in order of decreasing mass). The three largest RNAs are required to initiate infection. RNA 4 codes for the viral coat protein and arises during replication from RNA 3. Some CMV strains contain small satellite RNAs (110,000 M_r) that are unrelated to the viral RNAs but depend upon them for replication. Our laboratory has designated these satellites CARNA 5 for CMV-associated RNA 5 (18,20). Many different cucumoviral satellites have been characterized with respect to their nucleotide sequence (2,6,10,14,25), their ability to be translated in vitro (3,13,23), their effect on symptom development in a variety of different hosts (9,12,15,16,20,22,26,29), their effect on helper virus replication (7,12,17,24), and their transcription into biologically active CARNA 5 from cloned cDNA (5).

One of the most dramatic diseases caused by CMV strain D containing CARNA 5 is lethal stem necrosis of tomato (*Lycopersicon esculentum* Mill.). Stem necrosis is characterized by the following stages: about 8–10 days after inoculation of the cotyledons, necrotic flecks appear on the first leaves. Shortly thereafter, epinasty occurs and midrib and lateral veins become necrotic. Necrosis advances to the petiolules and to the petioles and

then to the stem until the entire top of the plant appears wilted, followed by drying of the tissue (20). The satellite D-CARNA 5 that causes this symptom has been characterized biochemically and sequenced (25). Recently we have begun to investigate a possible role of the genetic background of the host in the lethal stem necrosis symptom following CMV-D infection with D-CARNA 5. In contrast to many *L. esculentum* cultivars tested, several exotic *Lycopersicon* spp. respond with only mosaic symptoms to infection with CMV-D plus D-CARNA 5.

MATERIALS AND METHODS

Virus and plants. CMV strain D (21) and its CARNA 5 were propagated in *Nicotiana tabacum* L. 'Xanthi-nc'. Viral RNAs were isolated from purified virus preparations by phenol/SDS extraction followed by several ethanol precipitations. CARNA 5 was separated from genomic RNAs by several cycles of sucrose gradient ultracentrifugation (19).

Seeds for 96 *L. esculentum* cultivars (list available upon request) and *Lycopersicon* spp. were obtained from the USDA Regional Plant Introduction Station, Iowa State University, Ames. Preliminary screening of *Lycopersicon* spp. for their reaction to CMV-D infection was performed in the greenhouse during the winter and spring. Plants (usually eight) were mechanically inoculated with genomic RNAs at 10 μg/ml plus D-CARNA 5 at 2.5 μg/ml. Later experiments were performed in controlled environment chambers (10,000 lux, 16-hr day and 8-hr night).

Detection of viral and satellite RNAs. Viral and satellite RNAs were detected via dot-blot hybridization. Plant tissues were ground in buffer (50 mM Tris-HCl, pH 8.0, containing 1 mM EDTA) at 10 vol/g and held on ice until a fivefold dilution series in 20× SSPE was performed on all samples (20× SSPE = 3.6 M NaCl, 20 mM EDTA, 200 mM Na_2HPO_4, pH 7.7, with NaOH). Samples (50 μl) were applied to wetted nitrocellulose paper using a Bio-dot Microfiltration apparatus (Bio-Rad Laboratories, Richmond, CA), and samples were rinsed with 200 μl of 20× SSPE per sample. Air-dried filters were baked for at least 20 min at 80 C in vacuo. Prehybridization treatment, radioactive probing, and washing of the filters were as previously described (4). Plasmid pUC9 (28) containing a CARNA 5 insert (a gift from Dr. C. W. Collmer) was labeled using a protocol provided with the nick-translation kit obtained from Bethesda Research Laboratories (Gaithersburg, MD). cDNA probes to genomic RNAs were synthesized according to Taylor et al (27) as modified by Gould and Symons (11).

Polyacrylamide gel electrophoresis and sequence analysis. Purified CARNA 5 (0.5–1.0 μg) in 75% formamide, 8 M urea, 0.075% bromophenol blue, and xylene cyanol was boiled for 3 min and quenched in ice water; electrophoresis was performed for 16 hr at 10 V/cm on 9% acrylamide slab gel (39:1 acrylamide/bisacrylamide containing 8 M urea, 40 mM Tris-acetate, pH 7.8, plus 4 mM EDTA) (11.25 cm wide × 15.0 cm long × 0.8 mm deep) (28). RNA was visualized by ethidium bromide staining followed by ultraviolet photography.

Sucrose density gradient purified CARNA 5s were sequenced by the dideoxy method previously described (1) and modified to manufacturer's suggestion for replacing α-^{32}P-dATP with α-^{35}S-dATP (New England Nuclear, Boston, MA). Reverse-transcription reactions were primed with oligmer d(GGGTCCTG), which was manually synthesized by the phosphite triester method (New England BioLabs, Beverly, MA).

RESULTS AND DISCUSSION

All 96 *L. esculentum* cultivars inoculated with CMV-D containing D-CARNA 5 reacted necrotically (data not shown). Occasionally, one or two plants would show mosaic symptoms with no necrosis. These plants contained low levels of CARNA 5 as determined by dot-blot hybridization. Further testing of the cultivars showed this lack of necrosis to be either a rare event or the result of environmental factors (possibly temperature).

Several exotic *Lycopersicon* spp., however, did not respond necrotically when inoculated with CMV containing necrogenic D-CARNA 5 (Table 1). Many accessions of *L. hirsutum* Humb. & Bonpl., *L. hirsutum* f. *glabratum* Humb. & Bonpl., *L. parviflorum* C. M. Rick, and *L. chmiewlewskii* Riley reacted to viral infection with only mosaics. All accessions responding nonnecrotically contained CARNA 5 as determined by dot-blot hybridizations. All accessions were found to be susceptible to CMV and CARNA 5. To determine whether the infected accessions without necrosis contained a necrogenic CARNA 5, expressed leaf sap was used to inoculate Rutgers tomatoes. All inoculated Rutgers plants responded necrotically, suggesting that necrogenic CARNA 5 was present in accessions that developed only mosaic symptoms.

Several accessions were selected for further analysis. The yield of virus from several accessions ranged from 38 to 83% of the yield from CMV-infected Rutgers tomato (Table 2). The slight reduction in yields from the accessions may have resulted from the

TABLE 1. Percentage necrotic reaction of *Lycopersicon* spp. to infection with cucumber mosaic virus strain D containing D-CARNA 5

Species	Accession number	Necrotic plants (%)
L. cheesmanii	231257	0
	365896	100
	379035	0
	379039	100
L. chmiewlewskii	379030	0
L. esculentum cv. Rutgers		100
L. esculentum × *L. pimpinellifollium*	133542	100
L. hirsutum	126445	0
	127826	22
	128644	0
	209978	0
	308182	0
	365903	0
	365904	0
	365905	0
	365906	0
	365908	0
	379010	0
	379012	0
	379013	0
	379014	0
	390513	0
	390517	14
	390518	25
	390658	0
	390659	0
	390660	0
	390661	0
	390662	0
	415127	100
L. hirsutum f. *glabratum*	126449	0
	134417	10
	134418	0
	199381	0
	251304	25
	251305	0
	365907	0
	390514	14
	390516	0
L. parviflorum	379031	14
	379033	0
L. peruvianum	127832	100
	128650	40
	129152	45
	326173	32
	365969	67
	379029	8
	379032	0
	379034	67
L. pimpinellifollium	79532	100
	127805	100
	212409	100
	344102	100

difference in plant growth rates and from the fact that virus-infected Rutgers tomato plants were at initial stages of necrosis at harvest, which would lower the fresh weight of the tissue.

Purified satellite RNAs were characterized using semidenaturing polyacrylamide gel electrophoresis, which often separates CARNA 5 sequence variants (8). All CARNA 5s purified from accessions migrated to the same position as D-CARNA 5 used in the inoculum (Fig. 1). Partial sequence determination of CARNA 5s from accession 390661 (nucleotides 2–300) and 390518 (nucleotides 2–250) showed them to be identical to D-CARNA 5. The regions sequenced have previously shown sequence diversity in other CARNA 5 isolates (2,6,10,14,25).

These results show that many exotic *Lycopersicon* spp. replicate CMV and CARNA 5 efficiently and that the satellite progeny can induce necrosis in Rutgers tomato and appear to be identical in molecular structure to the CARNA 5 used in the inoculum. We are now attempting to determine the number of host gene(s) involved in this symptom.

TABLE 2. Virus yield from *Lycopersicon* spp. infected with cucumber mosaic virus strain D containing D-CARNA 5

Species	Yield (mg/kg)[a]
L. esculentum cv. Rutgers[b]	120
L. cheesmanii 231257	100
L. hirsutum 308182	46
L. hirsutum 390661	63
L. hirsutum 415127[b]	75
L. hirsutum f. *glabratum* 390516	63

[a] Average of two purifications.
[b] Plants showing stem necrosis.

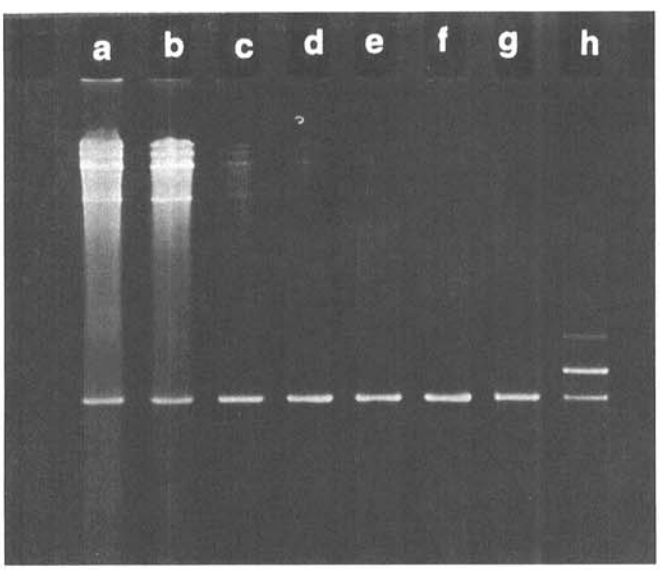

Fig. 1. Polyacrylamide gel electrophoretic characterization of CARNA 5s from infected *Lycopersicon* spp. Electrophoresis was on 9% gels under semidenaturing conditions (8). CARNA 5s from *L. hirsutum* 126445, 390518, 390661, 308182, and 415127 in lanes A–E, respectively; *L. chmiewlewskii* 379030 in lane F; *L. hirsutum* f. *glabratum* 390516 in lane G; and marker RNAs: nonnecrogenic 1-CARNA 5 (6), D-CARNA 5, and P—the satellite RNA from peanut stunt virus (18)—in lane H. Lanes C–G CARNA 5s were purified via sucrose gradient ultracentrifugation before analysis. Lanes A and B CARNA 5s were total RNA preparations from purified virions.

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