# Detection of Tomato Yellow Leaf Curl Virus in Lysates of Plants and Insects by Hybridization with a Viral DNA Probe

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**ABSTRACT**

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Tomato yellow leaf curl virus (TYLCV) was diagnosed in tomato plants and in insect vectors by use of a virus-specific probe. By molecular hybridization with radiolabeled TYLCV DNA, we detected viral nucleic acids in lysates prepared from infected tomato plant tissues such as leaves, roots, stems, shoot apex, and cotyledons and showed that the virus is not evenly distributed throughout the plant. The TYLCV probe also detected the virus in lysates of individual whitefly (*Bemisia tabaci*) vectors. The TYLCV-specific DNA probe can serve as a powerful tool in monitoring the tomato yellow leaf curl disease.

Additional keywords: diagnostics, geminivirus, plant viral disease

Tomato yellow leaf curl disease severely affects tomato (*Lycopersicon esculentum* Mill.) crops in eastern Mediterranean countries. This viral disease is transmitted by the whitefly *Bemisia tabaci* Genn. during summer and autumn (4); it is not transmissible mechanically (5). Symptoms similar to those characteristic of this disease have been described in North and Central Africa, Southeast Asia, and Taiwan (11).

All tomato cultivars currently available are susceptible to the disease. The insect vector is usually controlled, although not very efficiently, by frequent sprays of insecticides throughout the growing season.

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We have isolated the tomato yellow leaf curl virus (TYLCV) from infected tomato and jimsonweed (*Datura stramonium* L.) plants (6). The virus has a twinned particle morphology (20 × 30 nm) and a 2,800 nucleotide circular single-stranded DNA genome characteristic of the geminiviruses (9,10).

In this report, we show that by using purified TYLCV DNA as a probe in molecular hybridization tests, we can detect the presence of TYLCV nucleic acids in lysates prepared from infected tomato plant tissues (leaf, root, stem, cotyledon, shoot apex) and in lysates prepared from viruliferous insects.

## MATERIALS AND METHODS

**Maintenance of whiteflies and virus cultures.** Virus cultures were maintained in jimsonweed plants. Whiteflies were kept on cotton plants (*Gossypium hirsutum* L.) grown in muslin-covered cages held in an insectproof greenhouse. Virus was acquired by the whitefly vector after an access period of 18 hr on TYLCV-infected jimsonweed plants (4,5). Healthy tomato plants were inoculated at the five-leaf stage by access to viruliferous whiteflies for 24 hr. Tomato plants were grown subsequently in an insectproof greenhouse and sprayed biweekly with senprotathrin (Smash).

**Isolation of TYLCV and purification of viral genomic DNA.** Virus was prepared from plants 1–2 wk after the appearance of disease symptoms, by centrifugation of infected leaf extracts on sucrose gradients. DNA was extracted from virus preparations with phenol-chloroform, following a 2-hr incubation at 50 C in 0.4% sodium dodecyl sulfate (SDS) and 50 μg/ml of proteinase K (6).

**Preparation of lysates and DNA from tomato plants and from whiteflies.** Plant lysates were prepared by adapting the DNA extraction procedure previously described (16). Tomato tissues were frozen in liquid nitrogen and ground to a fine powder. Boiling extraction buffer (containing 2% [w/v] hexadecyltrimethyl-ammonium bromide [CTAB], 100 mM Tris-HCl [pH 8.0], 20 mM ethylene-diaminetetraacetic acid [EDTA], and 1.4 M NaCl) was added (1 ml/g of tissue). After 1% (v/v) 2-mercaptoethanol was added, the mixture was boiled for 1 min and incubated for 5 min at 55 C. Then, an equal volume of chloroform-isoamyl alcohol (24:1, v/v) was added, and the aqueous phase was extracted and collected after a 10-min centrifugation at 6,000 *g* (leaf lysate).

Insect lysates were prepared by squashing whiteflies in an Eppendorf tube in the presence of a minimum

volume of 100 μg/ml of proteinase K and 0.5% SDS. After a 2-hr incubation at 60 C, the mixture was centrifuged for 2 min at 6,000 *g* and the supernatant was collected (insect lysate).

Plant DNA was prepared according to the CTAB-based method (16), and whitefly DNA was prepared by use of the phenol extraction procedure (12).

**Gel electrophoresis of plant lysates and standardization of DNA amounts in lysates.** Lysates were electrophoresed in 1% (w/v) agarose gels containing 0.5 μg/ml of ethidium bromide, in Tris-phosphate-EDTA buffer (12). Tomato genomic DNA in a lysate migrated as a single band near the origin of the gel. DNA concentrations in tissue lysates were standardized in gels with known amounts of tomato genomic DNA.

**Molecular hybridization with radiolabeled TYLCV DNA.** After gel electrophoresis, samples were blotted onto nitrocellulose membranes (14). Blots were hybridized for 16 hr (3) with TYLCV DNA radiolabeled with $^{32}$P-dCTP (Amersham) by nick translation (13). Blots were then washed at 65 C for 30 min (twice) in 150 mM NaCl and 15 mM trisodium citrate (1 × SSC) before autoradiographic exposure using Kodak XAR-5 film.

**Dot-blot hybridization assay.** Samples were incubated for 30 min at 50 C in 0.5 N NaOH and 1.5 M NaCl, then neutralized with 1 N HCl. About 5 μl of each DNA solution was spotted on dry nitrocellulose membrane. After drying, the membrane was washed with 1 × SSC and baked for 2 hr at 80 C. Hybridization with radiolabeled TYLCV DNA and autoradiography were as described above.

## RESULTS

**Detection of TYLCV nucleic acid sequences in lysates prepared from TYLCV-infected tomato leaves.** Leaf lysates were prepared from healthy and from infected tomato plants. TYLCV DNA, although single-stranded, could be radiolabeled by nick translation (13), either because of local double-stranded stem structures similar to those described in genomic DNA of other geminiviruses (9,10) or because of contaminating plant DNA serving as primer. The lysates were submitted to gel electrophoresis, blotted, and hybridized with the radiolabeled viral DNA probe. Figure 1 shows the resulting autoradiogram. The TYLCV probe hybridized specifically with samples prepared from TYLCV-infected plants, never with lysates from healthy plants. Using known amounts of viral DNA, we could determine that the probe detects virus nucleic acid amounts as low as 10 pg (*data not shown*).

Lysates from tomato plants infected by other viruses affecting tomato, such as tobacco mosaic virus, cucumber mosaic virus, and potato virus Y, did not hybridize with the TYLCV probe (*data not shown*).

**Distribution of TYLCV nucleic acids in a TYLCV-infected plant.** The distribution of viral DNA sequences and their relative amounts in a TYLCV-infected plant were assessed. Lysates were prepared from a tomato plant when the first characteristic disease symptoms were recorded (about 2 wk after inoculation). Lysates were prepared from roots, cotyledons, shoot apex, leaves from different ages, and stems. Samples standardized to contain the same amount of plant genomic DNA (about 2 μg per sample) were submitted to gel electrophoresis, blotted, and hybridized with the TYLCV DNA probe. The results shown in Figure 2 indicate that the virus is not evenly distributed throughout the plant tissues. The highest concentration of viral genomic DNA (circular single-stranded [CSS]) was found in the shoot apex (lane 9). Much lower concentrations were detected in the roots (lane 1) and stems (lanes 3 and 5), and still lower concentrations were found in expanding leaves (lanes 6–8). Almost no viral DNA sequences could be detected in nondividing tissues, such as older leaves (lane 4) and cotyledons (lane 2).

In addition to the CSS viral genomic DNA, other virus-related DNA species were detected by the TYLCV DNA probe (Fig. 2). They have been identified as

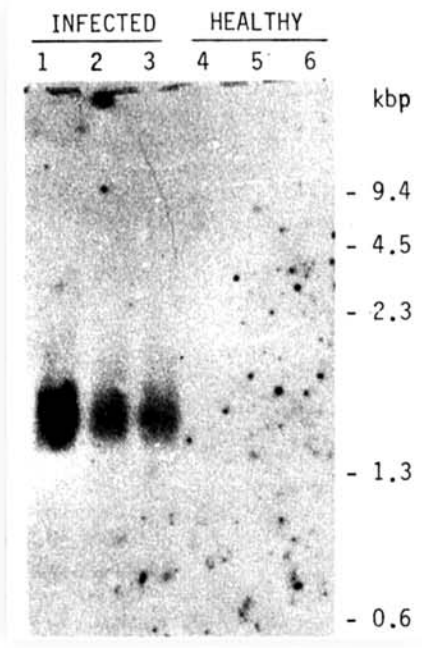


**Fig. 1.** Autoradiographic detection of tomato yellow leaf curl virus (TYLCV) nucleic acids in TYLCV-infected tomato leaf lysates. Lysates were prepared from the leaves of three infected tomato plants (lanes 1–3) showing typical disease symptoms (about 2 mo after inoculation). Lysates were also prepared from three healthy control tomato plants (lanes 4–6). The lysates were submitted to agarose gel electrophoresis, blotted, and hybridized with the radiolabeled TYLCV probe. Size markers (listed but not illustrated) on the right (in kilobase pairs [kbp]) were from a EcoR I/Hind III digest of bacteriophage lambda DNA.

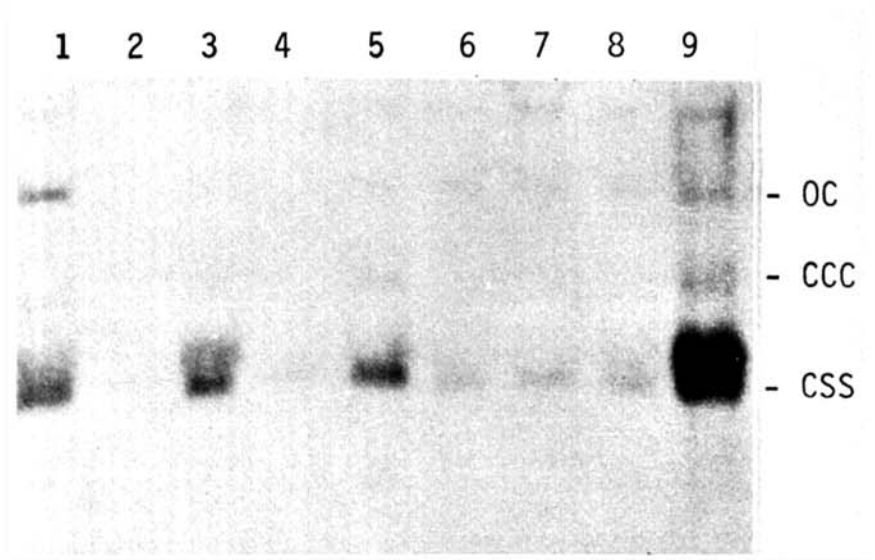


**Fig. 2.** Distribution of tomato yellow leaf curl virus (TYLCV) in the tissues of a tomato plant infected by viruliferous whiteflies in the greenhouse at the five-leaf stage and sampled 15 days after inoculation. At that time, nine leaves were present (numbered 1 to 9, from top to bottom); leaves 1–4 developed after inoculation. Lysates were prepared from roots (lane 1), cotyledons (lane 2), stem between leaves 8 and 9 (lane 3), leaves 8 and 9 (lane 4), stem between leaves 1 and 8 (lane 5), veins of leaves 4 and 5 (lane 6), leaves 4 and 5 (lane 7), leaves 1 and 2 (lane 8), and shoot apex (lane 9). The lysates were submitted to gel electrophoresis and blotted; blots were hybridized with the TYLCV probe and autoradiographed. CSS = circular single-stranded viral genomic DNA; CCC and OC = circular double-stranded DNA replicative form of the viral genome in its supercoiled (covalently closed circular [CCC]) and relaxed (open circular [OC]) forms.

being the circular double-stranded DNA replicative forms of the viral genome in its supercoiled (covalently closed circular [CCC]) and relaxed (open circular [OC]) forms (*unpublished*). These DNA forms are similar to those found in plants infected by either the tomato golden mosaic virus (8) or the cassava latent virus (15).

**Detection of TYLCV DNA sequences in lysates of viruliferous whiteflies.** Female whiteflies were kept on TYLCV-infected jimsonweed plants for an acquisition period of 18 hr (5), collected, and immediately frozen. Control whiteflies were kept on healthy plants. Lysates were prepared from 1, 5, and 50 insects and spotted onto a nitrocellulose membrane. DNA samples purified from 100 viruliferous and 100 control whiteflies were spotted also. Figure 3 shows the results of hybridization with the TYLCV DNA probe. It is evident that the probe can detect viral DNA sequences in a lysate prepared from an individual viruliferous whitefly. No hybridization signal was obtained with lysates prepared from control nonviruliferous whiteflies. The probe hybridized equally well to DNA purified from viruliferous whiteflies.

## DISCUSSION

Whitefly-transmitted tomato yellow leaf curl virus is the cause of a tomato disease that can lead to enormous crop losses. The disease seems to be spreading to regions where it was not reported before. Recently, for the first time in North America, a geminivirus has been isolated from tomato plants with TYLCV disease symptoms from Sonora, Mexico (2). Because satisfactory disease control is hard to achieve, the development of rapid and accurate detection tests based on the availability of TYLCV DNA probes may provide a valuable tool for monitoring virus epidemics.

The detection and diagnosis of whitefly-borne geminiviruses has relied for a long time on traditional methods, such as symptom expression and transmission to test plants (5,7). We have shown here that molecular hybridization with a virus-specific probe makes it possible to detect TYLCV nucleic acid sequences not only in lysates from infected plants but also in lysates of its whitefly vector.

TYLCV DNA was detected in lysates prepared from different tissues of infected tomato plants. The use of lysates provides an accurate estimation of virus concentration in these tissues, since it minimizes the nucleic acid losses inevitable in the process of DNA purification. Although the infection took place in cages where insects were free to inoculate whatever tissues were available during a 24-hr period, viral DNA was not evenly distributed throughout the infected plant tissues. Highest concentrations were found in tissues containing rapidly dividing cells, such as the roots and the shoot apex. Lowest concentrations were detected in mature tissues, such as cotyledons and leaves. These findings may indicate that the virus needs active cellular DNA synthesis to replicate its own genetic material.

Viral DNA could be detected as early as 4 days after inoculation, 10–20 days before the first characteristic disease symptoms appear (*unpublished*). It is possible that at this stage the concentration of virus is not sufficient to be effectively spread by insects. If so, it might be feasible to take advantage of this time lag to destroy infected plants in order to slow down or stop TYLCV epidemics.

TYLCV DNA sequences can be detected in extracts of individual viruliferous whiteflies. It has been shown previously that the leafhopper-transmitted maize streak virus, another geminivirus, can be detected in squashes of individual insects (1). Therefore, epidemiological studies could be conducted either by monitoring the spread of viruliferous insect vectors or by identifying virus-infected plants. A better understanding of the virus-insect-plant relationships may be a key point in developing means to prevent TYLCV infection or in breeding TYLCV-resistant tomato cultivars.

The methods described here could be applied to detect the presence of other whitefly-borne geminiviruses in the infected plant and in the insect vector, particularly those for which DNA probes are available.

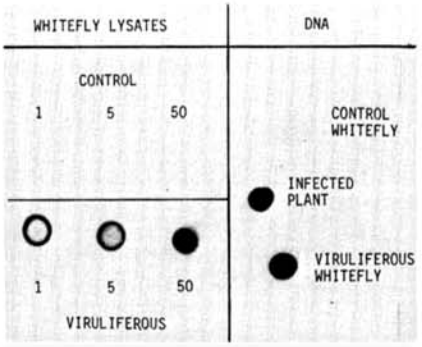


**Fig. 3.** Spot hybridization of lysates and DNA prepared from tomato plants and whiteflies. Left panel: Lysates prepared from 1, 5, and 50 whiteflies (control and viruliferous) were spotted on a nitrocellulose filter. Right panel: DNA prepared from 100 control and 100 viruliferous whiteflies and 3 $\mu$g of infected tomato plant DNA were spotted similarly. The membrane was hybridized with the tomato yellow leaf curl virus (TYLCV) DNA probe and autoradiographed.


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