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Serological Relationships between Tomato Yellow Leaf Curl Virus, Mung Bean Yellow Mosaic Virus and Tobacco Leaf Curl Virus

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ABSTRACT

An antiserum against tomato yellow leaf curl virus (TYLCV, Thai isolate) was prepared by immunizing a rabbit with purified virus preparation. The TYLCV antiserum obtained gave a single precipitin line and had a titer of 1/128 with purified virus in gel double diffusion tests. Serological tests indicated that TYLCV is serologically identical with tobacco leaf curl virus (TLCV, Japanese isolate). TYLCV was serologically distinquishable from mung bean yellow mosaic virus (MYMV, Thai isolate) by spur formation in gel double diffusion tests.

Keywords: geminivirus, tomato yellow leaf curl virus, tobacco leaf curl virus, mung bean yellow mosaic virus, whitefly-transmitted virus.

INTRODUCTION

A yellow leaf curl disease of tomato is one of the most serious diseases of tomato in Thailand (Thongrit, *et al.*, 1986). The causal agent of the disease, tomato yellow leaf curl virus (TYLCV), has been placed in the geminivirus group because of its geminate particles and a genome of covalently closed circular single stranded DNA (Attathom *et al.*, 1990). A leaf curl disease of tobacco has been reported from Japan, and many tropical and subtropical countries (Muniyappa, 1980; Osaki and Inouye, 1981; Honda, 1986). The causal agent of the disease, tobacco leaf curl virus (TLCV), has been identified as a member of geminivirus group (Osaki and Inouye, 1981). TYLCV and TLCV are transmitted by the same vector, *Bemisia tabaci* Genn., in a persistent manner. Symptoms caused by TLCV and TYLCV on tomato plants are similar.

In this paper we describe that TYLCV and TLCV are serologically identical. We have also studied serological relationship between two geminiviruses in Thailand, TYLCV and mung bean yellow mosaic virus (MYMV).

MATERIALS AND METHODS

Virus purification : TYLCV (Thai isolate) and TLCV (Japanese isolate) were maintained and propagated by graft inoculation into tomato plants, *Lycopersicon esculentum*, kept in an insectfree glass house. TYLCV was purified from infected tissues by the method previously described (Attathom, *et al.*, 1990). TLCV (Japanese isolate) and MYMV (Thai isolate) were purified from infected tobacco and mungbean plants according to the methods described by Ikegami *et al.* (1987) and Ikegami and Shimizu (1988), respectively.

Antiserum production : TYLCV antiserum was prepared by immunizing rabbits with four intramuscular injections of purified TYLCV (0.5 ml, concentration : 100 ug/ml) mixed with 0.5 ml Freund's complete adjuvant. The second injection was done 2 weeks later and followed by one week interval for the third and fourth injection. The antiserum to TYLCV was obtained one week after the final injection and stored at 4°C with 0.02% NaN_3. The antiserum to MYMV in our laboratory stocks had a titer of 1/512 in gel double diffusion tests (Ikegami and Shimizu, 1988).

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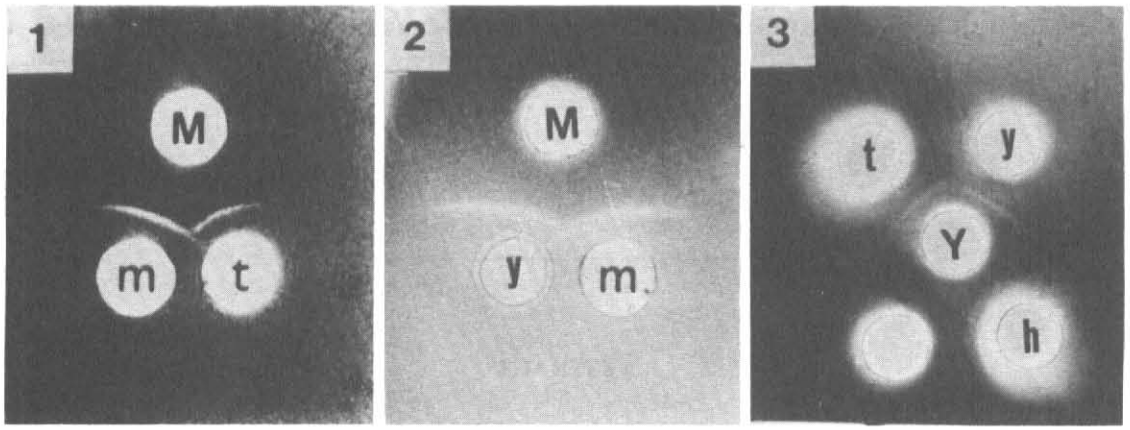

Figure 1) **Two-dimentional immunodiffusion of antiserum to MYMV (well M) with partially purified MYMV (well m) and partially purified TLCV (well t).**
2) **Two-dimentional immunodiffusion of antiserum to MYMV (well M) with partially purified MYMV particles (well m) and purified TYLCV (well y).**
3) **Two-dimensional immunodiffusion of antiserum to TYLCV (well Y) with partially purified TYLCV (well y) and TLCV (well t). Well h : healthy tomato extract.**

Gel double diffusion tests : Gel double diffusion tests were carried out as described by Ikegami and Shimizu (1988) with some modifications. Samples of healthy and diseased leaf tissues, each of 1 g were ground in 0.1 M sodium phosphate buffer, pH 7.8 (1 g/3 ml) and filtered. Crude saps were centrifuged at low speed and supernatants were further high speed centrifuged at about 250,000 g for 90 min. The pellets obtained were resuspended in 50 ul of 0.01M sodium phosphate buffer, pH 7.8 containing 10 mM EDTA, and used for gel double diffusion tests. Gel was composed of 0.15% gelrite, 0.85% NaCl, 0.02% NaN_3 and 0.2% $MgCl_2$ in 0.1 M Tris HCl buffer, pH 8.0. Antisera against viruses were used as concentrated solution or diluted 1/10. The reaction was kept at 37°C in moist chamber for about 24-72 h.

RESULTS AND DISCUSSION

When partially purified TLCV and MYMV were placed in adjacent wells and allowed to diffuse against the antiserum to MYMV, the precipitin lines joined with a spur developed (Fig. 1). Although TLCV and MYMV had a common antigenic determinant, they were serologically distinguishable by spur formation. A spur was also observed when partially purified TYLCV and MYMV were tested with the antiserum to MYMV, indicating that TYLCV and MYMV were related but not identical (Fig. 2).

The antiserum to TYLCV prepared in this study had a reaction end point of 1/128 in gel double diffusion test and did not react with sap from healthy tomato leaves (Fig. 3). When partially purified TYLCV and TLCV were allowed to diffuse against the antiserum to TYLCV. The precipitin lines completely joined, indicating that TYLCV and TLCV were serologically identical (Fig. 3).

TYLCV and TLCV differed from MYMV in host range (Honda and Ikegami, 1986; Muniyappa, 1980). MYMV is transmitted by mechanical inoculation (Honda and Ikegami, 1986), but TYLCV and TLCV are not (Osaki and Inouye, 1981; Thongrit *et al.*, 1986). Our serological tests also indicated that TYLCV was serologically distinquishable from MYMV. However, TYLCV and TLCV are serologically identical. Moreover, TYLCV and TLCV are both transmitted by whitefly causing leaf curling on tomato and tobacco plants, respectively. Further experiments on molecular characterization would be necessary to elucidate the relatedness of TYLCV and TLCV.

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