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Characterization of Nucleic Acid of Tomato Yellow Leaf Curl Virus

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ABSTRACT

Tomato yellow leaf curl virus (TYLCV) was successfully isolated from infected SVRDC-4 and Seeda tomato plants. Purification procedure consisted of the extraction of powderized infected tissues in tris-HCl buffer, clarification with chloroform or butanol, precipitation of the virus with polyethylene glycol, and virus purification through caesium sulfate centrifugation. The viral nucleic acid was isolated from purified virus preparation by phenol-chloroform extraction followed by ethanol precipitation. Positive reaction to diphenylamine test and sensitivity to DNAse of isolated nucleic acid suggested that it is DNA. Studies on hyperchromic shift of heat and formaldehyde treated preparations indicated that TYLCV-nucleic acid is single stranded DNA. Its molecular weight as estimated by 0.7-0.8 % agarose gel electrophoresis is 0.63 x 10^6 daltons.

INTRODUCTION

Tomato yellow leaf curl disease was first reported in Thailand in 1973 (Chandrasrikul,1973). However, the virus causal agent was demonstrated 10 years later (Thanapas *et al.* ,1983). Preliminary studies on tomato yellow leaf curl virus (TYLCV) indicated that it is a geminivirus, transmitted by the whitefly *Bemisia tabaci* Genn. (Cohen and Harpaz, 1964; Goodman, 1981; Thongrit *et al.*, 1986). Tomato yellow leaf curl disease is considered to be the most destructive disease of tomato in Thailand where there is no effective control measure available.

In this study we investigated the physical and biochemical properties of TYLCV nucleic acid as part of the development program for the control of this disease.

MATERIALS AND METHODS

Virus isolate : A severe isolate of tomato yellow leaf curl virus (TYLCV) was obtained from naturally infected tomato plants. The virus was propagated on one month old seedlings of Seeda tomato by tissue implantation technique (Matthew,1970). Inoculated seedlings were kept in a glasshouse at 25-28°C for 2-4 weeks. After that, infected leaves showing yellow leaf curl symptoms (Fig.1) were harvested and used as virus source for purification.

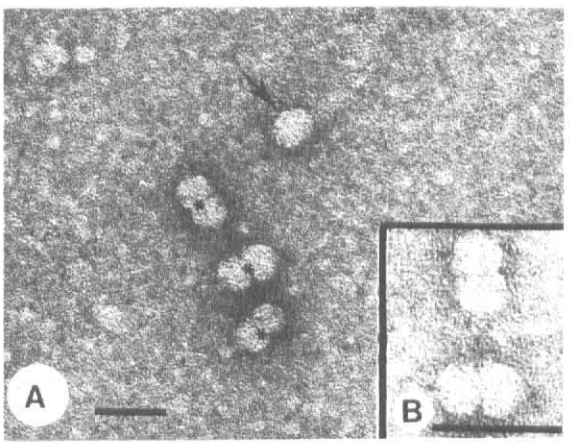

Fig.1 **A. Purified tomato yellow leaf curl virus (TYLCV) showing single (arrow) and geminate (asterisks) particles.**
B. Magnified particles in A. Bars represent 50 nm

Purification of TYLCV : The virus was purified from infected tomato leaves by the modification of the method described by Sequeira (Sequeira and Harrison., 1982). One kilogram of frozen tissues was homogenized in 2 litres of 0.1 M tris-HCl buffer, pH 8.4, containing 1 % 2-mercaptoethanol. The extract was

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filtered through 4 layers of cheesecloth and clarified with a litre of butanol or chloroform,and low speed centrifuged at 12,000 rpm for 10 min. The virus in aqueous phase was precipitated by adding 8% polyethylene glycol and 0.2 M NaCl, stirring for 90-120 min at 4°C and low speed centrifuged at 12,000 rpm for 10 min. The supernatant was subjected to high speed centrifugation at 45,000 rpm for 2 hrs. The pellet obtained was resuspended in 5mM tris-HCl buffer, pH 8.0 with 2.5mM EDTA, centrifuged at 12,000 rpm for 10 min and further purified by caesium sulfate gradient centrifugation at 50,000 rpm for 48 hrs. The virus zone was collected and dialyzed over night in 0.5 mM tris- HCl buffer.

Isolation of TYLCV nucleic acid : Purified virus adding with 0.1 % SDS was extracted with 1:1 (v:v) phenol-chloroform solution. The extraction was repeated twice. Nucleic acid in the aqueous phase was precipitated with 2.5 volume of absolute ethanol with 0.3 M sodium acetate, pH 4.8. After low speed centrifugation at 10,000 rpm for 10 min, the pellet was washed in 70% ethanol, vacuum dried and resuspended in TE buffer (0.1 M tris, 0.01 M EDTA) for further uses.

Physical - biochemical properties of TYLCV nucleic acid : The effect of heat on melting of TYLCV nucleic acid was determined by heating one ml of isolated nucleic acid for 10 min at various temperatures and their absorbances at 260 nm were recorded. Calf thymus DNA was used as control. Isolated TYLCV nucleic acid was treated with RNAse and DNAse, and analyzed on 0.7 % agarose gel electrophoresis. Lambda DNA cut with Hind III restriction endonuclease was used as markers. The gel was stained with 0.5 µg/ml ethidium bromide solution and viewed by a UV-illuminator.

Electron microscopy : Nucleic acid extracted from purified TYLCV was prepared for microscopic observation by using the method of protein- free spreading (Vollenweider, *et al.*, 1975). Isolated nucleic acid was mixed with benzyldimethyl alkylammonium chloride (BAC) solution to make the final concentration of nucletic acid and BAC to be 1 µg/ml and 2.5 x 10^3 g/ml, respectively. The mixed solution was dropped on parafilm sheet and left for 20 min. The nucleic acid film was picked up on carbon coated grids, washed with distilled water, dehydrated in ethanol and stained with uranyl acetate. The specimens were rotary shadowed with Pd-Pt wire and viewed under an electron microscope.

RESULTS

Virus purification : TYLCV was successfully purified from infected tomato tissues yielding 80 µg virus /kg tissue. Although the yield was not much different from previously reported (Thongrit *et al.*, 1986) virus preparation was clean and most of particles remained intact. Single and geminate particles with the average size of 18 and 18 x38.8 nm were observed in negatively stained preparation (Fig.2).

Viral nucleic acid : Nucleic acid extracted from

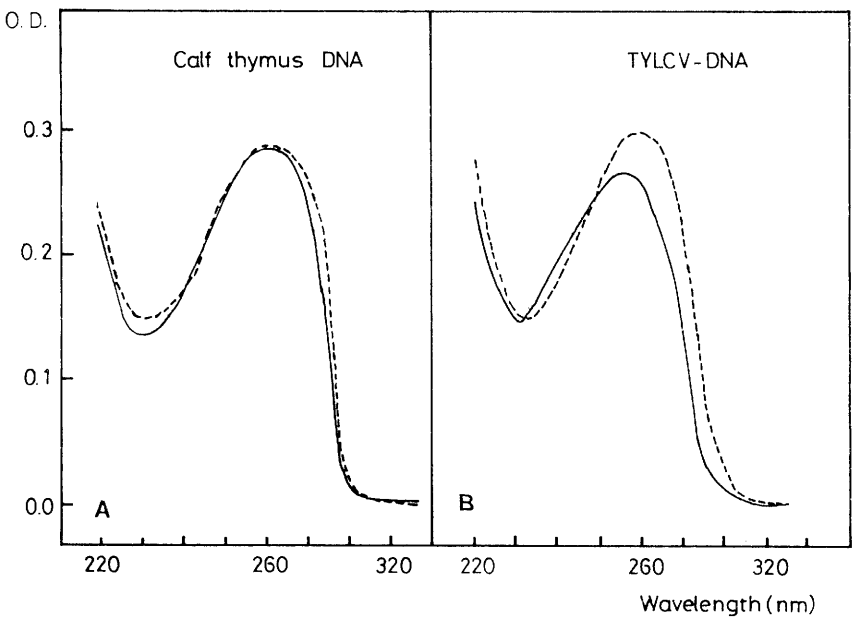

Fig.2 Effect of formaldehyde treatment on the UV absorbance of TYLCV-DNA (A), calf thymus DNA (B).

purified preparation of TYLCV showed typical absorbance profile of nucleic acid with A260/280 ratio of 1.85. Diphenylamine test of the isolated nucleic acid gave pale blue solution after reaction, resulting in an optical density of 0.6 at 595 nm. This result indicated that TYLCV possesses DNA as its genome. The effect of formaldehyde treatment on isolated nucleic acid was shown in Fig.3. It was found that maximum absorption of TYLCV-DNA increased about 11.3% after the treatment, and shifted from 257 to 260 nm. Minimum absorption also shifted from 233 to 235 nm. Heat denaturation of TYLCV-DNA resulted in hyperchromicity shift about 12 % with a typical profile of a single stranded nucleic acid as compared to calf thymus double stranded DNA, which showed a melting temperature at 84°c with hyperchromicity shift about 53%.

Estimation of molecular weight of TYLCV - DNA : Electrophoresis of extracted TYLCV-DNA on 0.7-0.8 % agarose gels always revealed two distinct bands of nucleic acid. Both of them were sensitive to DNAse but not RNAse (Fig.4). The estimated molecular weights of these DNAs were 1.19 x 10^6 daltons for the upper band and 0.6 x 10^6 daltons for the lower band. Electron microscopic observation of the eluted DNAs showed that the upper band was linear ss DNA, and the lower band was circular ss DNA (Fig.5). With the contour length of the circular ss DNA of 717 nm,

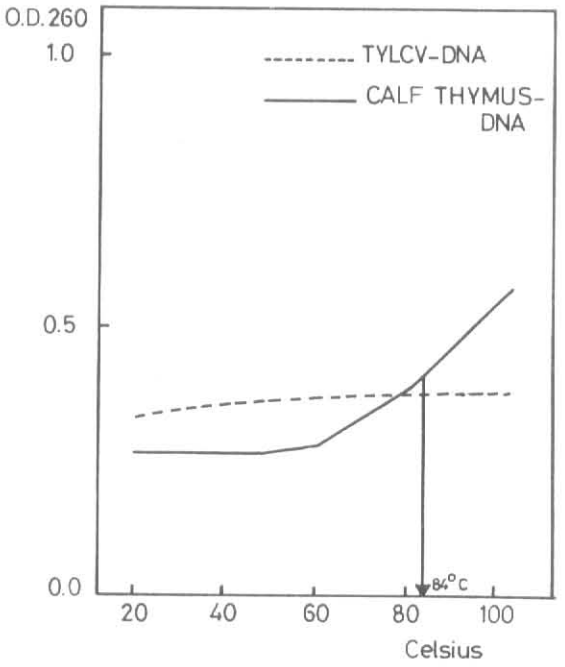

Fig.3 Hyperchromicity shift after heat denaturation (20-100° C) of TYLCV in 1 x SSC buffer.

and the use of ss DNA of ϕ174 as a control, it was estimated that TYLCV-DNA contained 2,001 nucleotides and its molecular weight was 0.63 x 10^6 daltons.

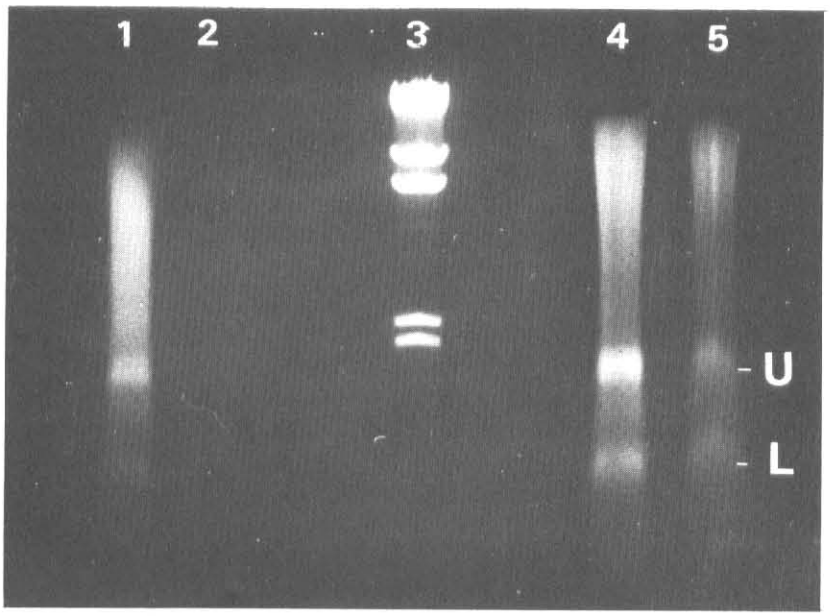

Fig.4 Agarose gel electrophoresis of TYLCV-DNA in TBE buffer. Lane 1,4:TYLCV-DNA, Lane 2;TYLCV-DNA treated with DNAse. Lane 5; TYLCV-DNA treated with RNAse. Lane 3;Fragments of Lambda DNA digested with Hind III (markers).
U = upper band L= lower band

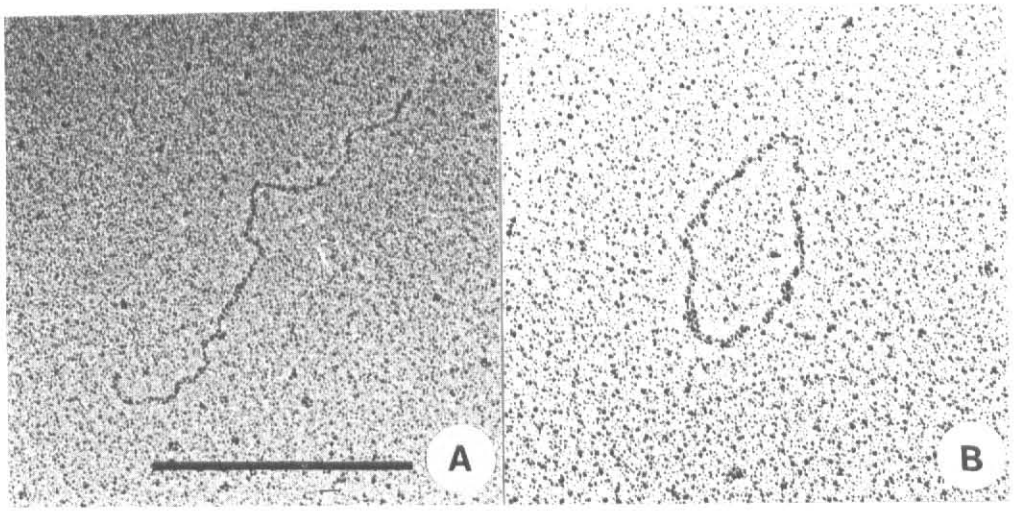

Fig.5 Electron micrographs of TYLCV-DNA eluted from agarose gel, followed by phenol extraction. A;linear form of DNA from upper band, B;circular form of DNA from lower band. Bar represents 500 nm.

DISCUSSION

This is the first report on physical and biochemical properties of TYLCV nucleic acid in Thailand. We have demonstrated the circular ss DNA of this virus as one of the important characteristics of the geminivirus where TYLCV belongs. However, the size of TYLCV circular DNA estimated by our study to be 0.63×10^6 daltons is somewhat lower than those previously reported of whitefly-transmitted geminiviruses of solanaceous plants like tobacco leaf curl virus (Ikegami *et al.* 1987), tobacco yellow dwarf virus (Thomas and Bowyer, 1980), tomato golden mosaic virus (Hamilton *et al.*, 1982) and tomato yellow leaf curl virus (Czosnek *et al.*, 1988). In most cases the molecular weights of circular ss DNA of geminiviruses are between 0.7-0.8 x 10^6 daltons. The existence of linear ss DNA of TYLCV is not well understood. It may be nicked circular molecule resulting from phenol extraction procedure. Moreover, the infectivity of both circular and linear ss DNAs of TYLCV will have to be determined.

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