

Purification of *Tomato leaf curl Bangalore virus* and production of polyclonal antibodies

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***Tomato leaf curl Bangalore virus* (ToLCBV) was successfully transmitted using viruliferous whiteflies (*Bemisia tabaci*) to several tomato varieties and hybrids, viz. Arka vikas, Pusa ruby, Rashmi, Rakshitha and Swaraksha. Hybrid Rashmi took 100% uniform infection, which was selected for further propagation of the virus. ToLCBV was also transmitted to *Nicotiana benthamiana*; the rate of infection was 30–40% in spite of two inoculations with 10–15 viruliferous *B. tabaci*/plant. The virus was purified and polyclonal antisera were produced in rabbits by injecting purified native virus and recombinant coat protein (rCP) of ToLCBV. The antisera of ToLCBV and ToLCBV rCP had a titre of 1 : 8000 and 1 : 400 respectively, when tested with infected leaf extract of tomato. Both sources of antisera successfully detected begomovirus infections in reservoir host plants, viz. *Parthenium hysterophorus*, *Acanthospermum hispidum*, *Ageratum conyzoides*, and field-collected tomato samples and cross-reacted with hibiscus leaf curl in DAC–ELISA.**

TOMATO leaf curl virus disease is one of the most destructive diseases, of tomato crop¹. In Karnataka 100% infection was observed in summer months, causing yield losses^{2,3} ranging from 27 to 90%. A reliable and accurate detection of virus is a pre-requisite to develop disease management strategies^{4,5}. For diagnostics, symptomatology does not provide a sound parameter and takes longer to establish the identity of the pathogen involved. Widespread application of immunological assays using polyclonal antisera has been utilized for decades in the identification of viruses^{6,7}.

The increasing importance of *Tomato leaf curl virus* has resulted in the need for accurate detection and identification procedures, stimulating intensive research efforts⁸. Detection is necessary to enable virus reservoir hosts to be identified, virus resistant plant varieties to be evaluated and consequently control measures to be devised or improved^{9,10}. Indian *Tomato leaf curl virus* was partially purified and its epitope variability was studied using a panel of monoclonal antibodies to *Indian cassava mosaic virus* (ICMV)⁸. However, there has been no report on production of polyclonal antisera to this important geminivirus.

Stock culture of *Tomato leaf curl Bangalore virus* (ToLCBV) was maintained in glasshouse on tomato cultivars Arka vikas or Pusa ruby by frequently inoculating 10–12-day-old tomato seedlings with viruliferous whiteflies. Whitefly *Bemisia tabaci* was maintained on cotton (*Gossypium hirsutum* cv. Laxmi) plants in wooden cages and used for inoculations.

About 400–500 young seedlings of tomato hybrid Rashmi were inoculated with ToLCBV to harvest leaf material for virus purification. The virus was purified by essentially following the protocol described by Muniyappa *et al.*⁸ with modifications consisting of purification from chloroform-clarified extracts in sodium citrate buffer by precipitation with polyethylene glycol, ultra centrifugation and sucrose density gradient centrifugation. Final pellet was resuspended in about 250–500 µl 0.1 M sodium citrate buffer and tested for the presence of virus by TAS–ELISA and used for further experiments.

About 250 µg of purified ToLCBV was emulsified in Freund's complete adjuvant and used for immunization of rabbits (strain New Zealand white) to produce polyclonal antiserum. After the final booster, animal was bled and serum was separated by keeping the blood at 4°C in a refrigerator overnight.

The coat protein gene of ToLCBV was cloned into PQE-30 vector and over-expressed in *Escherichia coli*, as described by Kirthi and Savithri¹¹. Purified recombinant coat protein (rCP) of ToLCBV run on 10% SDS–PAGE was stained with Coomassie blue. Protein band (~250 µg) of 35 kDa corresponding to ToLCBV was cut, ground thoroughly to make a fine paste and injected into rabbit at multiple sites to produce polyclonal antiserum, as detailed earlier.

Polyclonal antiserum produced to intact native virus was cross-adsorbed with healthy tomato leaf material extracted in PBS-T containing polyvinyl pyrrolidone and ovalbumin (PBST–PVP–OA), whereas antiserum raised to ToLCBV rCP was directly used for virus detection.

A direct antigen coating method (DAC–ELISA) as described in the literature¹² was used to determine the titre of antisera and subsequent detection of virus in host plants. Rabbit Fc-specific globulin prepared in goat (Sigma, USA) conjugated to alkaline phosphatase enzyme was used. The antigen sample was prepared by homogenizing the infected/healthy tissue in carbonate buffer (1 g/10 ml) containing 0.05 M diethyldithiocarbamate and used for coating the wells of ELISA plates. During field survey, *Tomato leaf curl virus*-infected samples, suspected samples and weeds were collected and tested for the presence of virus by DAC–ELISA.

ToLCBV was successfully purified from laboratory-inoculated tomato and *N. benthamiana* leaves collected after four weeks of inoculation. Concentration of the virus was higher in infected *N. benthamiana* (3.20 OD) compared to that of tomato (2.79 OD). The final purified virus reacted positively in TAS–ELISA using heterologous ICMV monoclonal antibodies (SCR 60).

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Table 1. ELISA-based survey for detection of virus in infected tomato, weeds and other geminivirus hosts collected from different locations in Karnataka

Place of collection	Variety/hybrid	Absorbance values A405 ^{a,b,c}	
		ToLCBV antiserum (1 : 1000)	TLCBV rCP antiserum (1 : 400)
Bangalore South district			
Hirendahalli	Avinash	2.04	1.10
	Arkavikas	1.55	0.80
	Namdhari	1.20	0.68
Guttahalli	Swaraksha selection	1.28	0.73
	Sun 176	1.45	0.79
	Swaraksha	1.18	0.64
Kodi	Nidhi	1.41	0.65
Kolar district			
Kencharlahalli	Rakshita	1.60	1.02
Kaiwara	Maharaja	1.90	1.15
Talagavara	Vaishali	1.80	0.98
Mindigal	Rashmi	2.00	1.20
H-cross	Avinash	1.80	0.96
Dharwad district			
Novalgund	Arka alok	1.95	1.00
Annigeri	Pusa ruby	1.60	0.84
Monigetti	Lerica	1.40	0.78
Alagawadi	Ramya	1.70	0.83
Davangere district			
Navilehala	Falguana	2.30	1.30
Daddaghatta	Roopali	2.10	1.11
Nyamathi	Namdhari	1.40	0.69
Shanthisagara	Pusa ruby	1.80	0.96
Weed samples ^d			
	<i>Parthenium hysterophorus</i>	0.91	0.75
	<i>Acanthospermum hispidum</i>	0.62	0.55
	<i>Ageratum conyzoides</i>	1.12	1.04
Other geminivirus ^e			
	Hibiscus leaf curl	1.09	0.70
Healthy	–	0.21	0.20
Buffer	–	0.08	0.08

^aTested in DAC-ELISA; ^bTwice the healthy is considered positive; ^cAverage of two wells; ^dSamples collected in tomato fields at UAS Hebbal, Bangalore; ^eSample collected at Chintamani, Kolar district.

Cross-adsorbed polyclonal antiserum raised against native ToLCBV was subjected to serial double dilutions, viz. 1 : 500, 1000, 2000, 4000, 8000, 16,000 and 32,000 in PBST-PVP-OA. Infected tomato leaf material extracted in carbonate buffer was also subjected to serial dilutions, viz. 1 : 5, 10, 100, 1000, 10000 and tested in DAC-ELISA. The antiserum had a titre of 1/8000, when tested with infected crude extract at 1 : 1000 dilutions.

Polyclonal antiserum was also successfully produced in rabbits against the bands of rCP of ToLCBV. Twofold dilutions of antiserum, viz. 1 : 200, 400, 800, 1600, 3200, 6400 were prepared in PBST-PVP-OA and tested in DAC-ELISA, as described earlier. The antiserum had a titre of 1/400 when tested with infected crude extract as antigen at 1 : 1000 dilution.

DAC-ELISA was standardized to detect ToLCBV in infected samples. During field survey, *Tomato leaf curl virus*-infected and suspected leaf samples were collected

from different locations in Karnataka and both ToLCBV and ToLCBV rCP antisera were able to detect virus in infected field samples.

Both sources of antisera were successfully employed to detect begomovirus infections in reservoir host plants, viz. *Parthenium hysterophorus*, *Acanthospermum hispidum* and *Ageratum conyzoides*. Among the different host plants tested that are infected with other geminiviruses, both sources of antisera successfully cross-reacted with hibiscus leaf curl samples collected from infected orchards. Muniyappa *et al.*⁸ used heterologous monoclonal antibodies raised against coat protein of ICMV and *African cassava mosaic virus* to detect *Tomato leaf curl virus* infections in field-collected tomato samples, weeds and whiteflies using TAS-ELISA.

ELISA-based survey was also conducted to assess the prevalence of *Tomato leaf curl virus* in tomato-growing areas of Karnataka. The per cent incidence was almost 100

in some of the fields surveyed, for instance, several commercial hybrids from Kolar district. Testing the leaf samples using indirect DAC–ELISA revealed the varying levels of virus concentrations. ToLCBV antiserum was more efficient than ToLCBV rCP antiserum in detection of virus in field collected samples (Table 1) Devaraja *et al.*¹³ successfully produced monoclonal antibodies to ToLCBV and detected begomovirus infections in tomato samples, and other crop and weed species.

Polyclonal antibodies produced using purified intact virus and rCP of ToLCBV presented here clearly demonstrate the utility of the antisera in begomovirus detection in field samples and reservoir hosts.

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