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–ELSIA.**

TOMATO leaf curl virus disease is one of the most destructive diseases, of tomato crop[1]. 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 efforts[8]. 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.

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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 (*Gossipium 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 Savithri[11]. 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 pyrolodone 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 literature[12] 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 diethyldithiocarbomate 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).

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

		Absorbance values A405[a,b,c]	
Place of collection	Variety/hybrid	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]			
	Parthenium hysterophorus	0.91	0.75
	Acanthospermum hispidum	0.62	0.55
	Ageratum conyzoides	1.12	1.04
Other geminivirus[e]			
	Hibiscus leaf curl	1.09	0.70
Healthy	–	0.21	0.20
Buffer	–	0.08	0.08

[a]Tested in DAC–ELISA; [b]Twice the healthy is considered positive; [c]Average of two wells; [d]Samples collected in tomato fields at UAS Hebbal, Bangalore; [e] Sample 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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Ground penetrating radar studies of a point-bar in the Mahi River Basin, Gujarat

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The present communication is an attempt to study a point-bar deposit using Ground Penetrating Radar (GPR). The vertical as well as lateral variations in the lithofacies and the continuity of the bounding surfaces are delineated using the trench and GPR profiles. The basal part of the point-bar at Angad indicates deposition under a near-continuous flow with minor changes in velocity and direction giving rise to fining upward planar cross-stratified sand facies typical of a point-bar sequence. However, the sediments in the upper part show high degree of lateral and vertical heterogeneity in terms of facies development and is inferred to have resulted due to deposition by high stage flood events associated with cyclonic storms and the gradual waning flow. The lithofacies assemblage and morphology of the bar from head to tail-end suggest that the bar deposition is mainly controlled by downstream accretion. The sediment sequences of the point-bar exposed in trenches and on GPR profiles are helpful in deciphering the hydrologic regime of the depositing river channel.

THE Mahi River arises in the Malwa Plateau, Madhya Pradesh near Moripara and flows through the uplands and alluvial plain in Gujarat before debouching into the Gulf of Cambay (Figure 1). In the alluvial plain, it shows typical meandering river morphology and is characterised by the presence of extensive point-bars along its convex meander bends. Point-bar deposits accumulate in response to lateral and/or downstream migration of the river channel. The ability of the river to move and deposit sediment depends on stream competence, stream power, velocity of water, availability and size of sediments at peak discharge. Moreover, the flow conditions in fluvial systems having monsoon variability are complex as for the Mahi River. In such cases, the study of point-bar deposits becomes important as the fluctuations in the near-channel water level are manifested in the form of distinct sediment sequences. The variations in lithofacies in a point-bar complex can be related to the variability in discharge of the river in the near past, that is a function of seasonal flood events. In this context, these modern river deposits are well-studied elsewhere[1–4], however, in India, studies on these deposits have been initiated recently[5–9]. The basic hindrance in establishing the stratigraphy of a point-bar is the lack of lateral continuity in marking the lithofacies and characteristic bounding surfaces, since sections can be studied along trenches in a limited area. This problem can

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