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SHORT COMMUNICATION

VALIDATION OF LOCALLY PRODUCED ANTISERA FOR THE IDENTIFICATION OF VIRUS AND VIRUS-LIKE DISEASES

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INTRODUCTION

Virus and virus-like organisms cause devastating damages to the cultivated crops causing severe economic losses to both farmers and the country. In order to conduct sensitive serological tests for rapid and accurate identification, antisera for the specific viruses or other pathogens are needed. Commercially available test kits may cost more than Rs. 100,000.00 (approx 760 US$), which can only be used to test less than 250 samples. The high cost, unavailability of commercial test kits for some locally important viruses, and the presence of restricted virus strains have made the production of local antisera test kits vital and indispensible.

The antisera production programme was initiated locally in 1990 at the Fruit Division of the Bombuwela Regional Agricultural Research Station, Sri Lanka. A local protocol for Enzyme-Linked Immunosorbent Assay (ELISA) was also developed subsequently (Dassanayake *et al.*, 1994a; Dassanayake *et al.*, 1994b; Dassanayake, 1997; Dassanayake and Rathnabharathi, 2002). As the production of the last stock of antisera was conducted at the beginning of the first decade of 21st century, the strength of the antiserum has come under question with time and storage conditions. Hence, this study was conducted to evaluate the sensitivity of locally produced antisera for different viruses to assess their reliability of use after long periods of storage.

MATERIALS AND METHODS

This study was conducted at the Plant Virus Indexing Centre (PVIC), Homagama and the Department of Microbiology of the University of Kelaniya, Sri Lanka in 2011 to evaluate the sensitivity of locally produced antisera for Banana Bunchy Top Virus (BBTV), Pineapple Wilt Virus (PWV), Papaya Ring Spot Virus (PRSV) and Citrus Greening Bacteria (CGB). These antisera have been produced in the year 2003 and 2005 and stored under refrigerated conditions (4 °C). The suspected diseased and healthy samples were tested for respective diseases using

locally developed indirect ELISA protocols as described by Dassanayake *et al.* (1994b) and Dassanayake (1997) with minor modifications. The original protocol suggests using antisera dilution of 1:2000 – 1:3000 due to their high detection level however, in the present study, a dilution of 1:100 was used as the antisera titre has reduced with time. The ELISA plate absorbance was read at 405 nm after incubating at 37 °C for one hour and over night. The success rate was calculated comparing the ELISA absorbance values and further confirmation was done through PCR-based using the protocols suggested by Su (1999) and Thomson *et al.* (1996) with minor modifications.

RESULTS AND DISCUSSION

Validation of locally produced ELISA test kits for BBTV and PRSV

The suspected diseased samples gave relatively higher absorbance values than those of the suspected healthy samples. The absorbance values increased with increasing degree of symptoms. The colour development was substantial in most diseased samples and a considerable colour reaction was shown within an hour after addition of the substrate. The reconfirmation also proved that the results given by ELISA are reliable (Table 1).

Table 1. Success rate of different antisrum

Antiserum tested for	*Number of samples*		*Reconfirmation methodology*	****Success rate from ELISA*
	*Tested (**diseased/ healthy)*	*Confirmed by ELISA*		
*BBTV	Diseased 20	20	Molecular-based techniques	100%
	Healthy 20	20	Molecular-based techniques	
PRSV	Diseased 20	20	Symptomatology	100%
	Healthy 20	20	Symptomatology	
PWV	Diseased 12	12	Molecular-based techniques	Not successful
	Healthy 12	0	Molecular-based techniques	
CGB	Diseased 6	0	Symptomatology	Not successful
	Healthy 6	0	Symptomatology	

*BBTV - Banana Bunchy Top Virus, PWV - Pineapple Wilt Virus, PRSV - Papaya Ring Spot Virus, CGB - Citrus Greening Bacteria;

**Samples were categorized as diseased or healthy through symptomatology of the sample;

***Success rate was calculated comparing the ELISA plate readings with the results through other methods

Validation of locally produced ELISA test kits for PWV and CGB

The colour reaction was observed in some wells. All the samples failed to produce a significant colour within an hour in both tests and the absorbance values after incubating at 37 °C for one hour were not significant. In fact, the absorbance values of CGB-test were distinguishable between healthy and diseased samples. All the samples tested for PWV were negative for the virus and none of the healthy samples were detected by ELISA. Therefore, both results failed to build a correlation with ELISA reading (Table 1).

CONCLUSIONS

The local ELISA test kits for BBTV and PRSV can be used efficiently for the identification of respective pathogens at least for five years after production under suitable storage condition. However, for sensitive detection of diseases, it is recommended that the antisera be produced at least once in 3 years. The ELISA test kits produced for PWV and CGB in this study were not reliable after long storage period and hence further investigations are necessary to reproduce the antisera for the respective diseases.

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