Annals of the Sri Lanka Department of Agriculture, 2002. 4:313-318

IDENTIFICATION AND DETECTION OF CASSAVA MOSAIC VIRUS IN CASSAVA

W.G.S. PERERA and E.M. DASSANAYAKE
Plant Virus Indexing Centre, Gabadawatte, Homagama

ABSTRACT

Cassava Mosaic Virus (CaMV) commonly found in the Western Province of Sri Lanka is a sap transmissible virus. It produces slight leaf deformation in cultivated cassava (*Manihot esculanta).* This virus induced single local lesions in *Chenopodium quinoa, Nicotiana* spp., *Physalis foetidana, Tetragonia expansa* and *Manihot esculanta.* Systemic reactions were found in *Nicotiana* spp and *Manihot esculanta.* It was possible to transmit the virus from diseased to healthy cassava seedlings at cotyledon stage by sap transmission but not to rooted cuttings. Furthermore, the virus can be transmitted through chip budding of cassava cuttings. Infectivity of crude sap of leaves was lost by heating at 45- 50^0 C for 10 minutes, by diluting 10^{-2}- 10^{-3} and by aging at room temperature for 4- 6-d.The virus was purified using young cassava leaves with symptoms, extracted in 0.1 M Tris HCl, pH 8.4, + 0.2% Monothioglycerol and clarified with half the volume of Chloroform. It was precipitated with 4%· PEG (MW 6000) and purified by two cycles of differential centrifugation. Partially purified virus was used to immunize a New Zealand white rabbit for the production of polyclonal antiserum. An antiserum dilution of 1: 4000 has the capacity to detect diseased samples by indirect enzyme linked immunosorbent assay

KEY WORDS: Cassava Mosaic Virus, Polyclonal Antiserum, Enzyme Linked Immunosorbent Assay.

INTRODUCTION

Cassava (*Manihot esculenta*) is susceptible to several virus diseases. It is reported to be infected by potyvirus, (Lennon *et al.*, 1986), potexvirus (Costa and Kitajima, 1972), *Carlavirus* (Lennon *et al.*, 1986) and *Gemini viruses* (Bock, Guthrie and Meredith, 1978). A gemini virus related to Indian cassava mosaic virus was detected by Salim and Bandumala (personal communication) in different isolates collected from cassava cultivations in Sri Lanka. Cassava plants with yellow mosaic symptoms and slight leaf deformation, is very common in edible and wild cassava cultivations. This crop is popular in lower income groups in many countries. Due to the virus infection yield reductions are experienced by cassava growers. Since the crop is propagated vegetatively, the chances are high for it to spread to uninfected areas. Therefore, it is important to identify and characterize virus infections in cassava crops. Further, it is of vital importance that suitable detection methods are available to differentiate diseased from healthy plants. The work reported here describes the partial characterization and detection of cassava mosaic virus by serological means.

MATERIALS AND METHODS

Virus isolates

Virus isolate was collected from a cassava cultivation in Biyagama, Sri Lanka. Cuttings were taken and propagated under aphid protected house at Plant Virus Indexing Centre, Gabadawatte, Homagama. Other isolates were collected from cassava growing areas in Kaduwela, Panagoda, Habarakada, Homagama,

Piliyandala, Maradana, Polgahawela and Alawwa. For the partial characterization, an isolate collected from Biyagama was used.

Host range studies

Young cassava leaves with symptoms were selected for virus extraction. Leaf sample was ground in a mortar with a pestle using 1/15 M Potassium phosphate buffer at pH 7.1 at a dilution of 1:10 w/v. The crude sap extract was inoculated to different plant species (Table 1). Carborandum (400 mesh) was used as an abrasive. Plants were briefly rinsed and covered with damp newspaper overnight. Following morning covers were removed and the plants were kept under aphid protected house for symptom development.

Graft transmission

Infected cassava tissues were removed and grafted to apparently healthy cassava and castor *(Weta endaru)* plants. Chip grafting was practiced All the grafts were maintained under aphid protected house for symptom expression. Results are given in Table 2.

Physical properties

Physical properties were determined according to standard method described by Noordam, 1973. Sap of cassava extracted with 1/15 M Potassium phosphate buffer, pH 7.1, was used to evaluate the physical properties.

Thermal inactivation point (TIP): Sap was extracted with 1/15 M potassium phosphate buffer pH 7.1 from infected cassava leaves and diluted 1:10 w/v with the same buffer. Two ml of sap was then pipetted into small tubes which were subjected to a temperature gradient from 40- 60^0 C for 10 minutes and immediately immersed in ice. The treated sap was mechanically inoculated several *Nicotiana glutinosa* plants. The numbers of local lesions were counted 19 days after inoculation, to investigate the thermal inactivation point. Results are given in Table 3.

Dilution end point (DEP): Concentrated crude sap as subjected to a series of 6 tenfold dilutions in 1/15 M Phosphate buffer pH 7.1. Each dilution was immediately inoculated on *N. glutinosa* test plants. The numbers of local lesions were counted 19 days after inoculation. Results are given in Table 3.

Longevity *in vitro* (LIV): The sap was extracted with potassium phosphate buffer pH 7.1 and diluted at 1:10 ratio. 1.5 ml of sap was pipetted into eppendorf tubes and stored at air conditioned room temperature (24^0 C). The sap was inoculated to*N. glutinosa* test plants at one day intervals for 9-d. Numbers of local lesions were counted 19-d after inoculation. Results are given in Table 3.

Virus purification

Systemically infected cassava leaves (Biyagama isolate), were used for the purification of the virus. Procedure similar to Sequeira and Harrison, (1982) with minor modifications was followed. Infected leaves (100g) frozen at -20^0 C for 4 days were homogenized with 400 ml of 0.1 M Tris HCl buffer pH 8.4, 0.2% monothioglycerol in a kitchen blender. Then the sap was squeezed through a

muslin cloth. For this sap extract, half volume of chloroform was added and stirred for 10 minutes in an ice bath. Clarified sap was then centrifuged at 8000 rpm at 4^0 C for 20 minutes. Supernatant was collected and 0.2 M NaCl + 4% PEG (MW 6000) was added. Then the extract was stirred for 90 minutes in an ice bath. Sap was then centrifuged at 19,000 rpm at 4^0 C for 90 minutes. Pellet was collected and resuspended in 0.01 M TE buffer, pH 8.0 + 0.005 M EDTA and stored at 4^0 C overnight. Then the resuspended pellet was homogenized for several minutes and centrifuged at 2000 rpm at 4^0 C for 5 minutes. Supernatant was collected. It was then centrifuged at 40,000 rpm at 4^0 C for 60 minutes. Pellet was collected and resuspended in 0.01 M TE buffer pH 8.0.

Final purification

Sucrose density gradient centrifugation (10%- 40%) 25,000 rpm for 2 _ h was done to separate out the virus zone. In addition differential centrifugation was also practiced for further purification.

Antiserum production

Partially purified virus was used. New Zealand white rabbit was initially injected with 0.5 ml virus preparation, mixed with an equal volume of .85% saline as intra-veinous (IV) to ear vein. Further 5 intra-muscular (IM) injections (1 ml) were given one week apart mixed with an equal volume of Fruends incomplete adjuvant. Bleeds were taken at 3 weeks and 6 weeks after immunization. The serum was separated after overnight incubation at room temperature. Enzyme linked immunosorbent assay test was done to determine the efficacy of the produced polyclonal antiserum.

Serological investigations

Antiserum absorption: As partially purified sap was utilized for antibody production, absorption was done to remove any antibodies of plant protein present in the serum. Apparently healthy sap was extracted by grinding in a mortar with pestle. Then the extracted sap was mixed with an equal volume of antiserum and incubated at room temperature for 3-h. After incubation, serum was centrifuged at 7000 rpm for 20 minutes. Supernatant was collected and this absorbed antiserum was used for serological investigations.

Enzyme Linked Immunosorbent Assay: Indirect ELISA was done according to Mowat and Dawson, 1987. Crude sap was extracted with two different buffers separately. Dilution factor was 1:10 w/v. Antiserum was diluted at 1:2000 in PBS-TPO buffer (Phosphate buffer saline 2 g/l Tween 20, 2% PVP, 0.2% Ovalbumin) Protein A conjugate dilution was 1:2000 in PBS-TPO. For each step standard washing steps were followed. Plate was read 1 hour after substrate addition. Results are given in Table 4. Efficacy of detection of polyclonal antiserum was done by these investigations.

RESULTS AND DISCUSSION

Results showed that the virus induced single local lesions in *Chenapodium quinoa, C.amaranticolor, Physalis foetidana, Nicotiana benthamina, N.glutinosa N.debneyi, N.tabacum Hawana, Manihot esculenta* (seedlings), and *Tetragonia expensa.* Systemic reactions were found in *Nicotiana* spp and *Euphobaceae* spp. Saunderd *et al.* (2002), reported that it is not possible to transmit cassava mosaic virus from cassava to cassava by sap transmission. However in the present study, when *Manihot esculenta* seedlings at cotyledon stage were used the virus was transmitted.

Furthermore the host range result according to them is different for the isolate under study in the present investigation. Those isolates produced systemic reactions in *Nicotiana glutinosa, N.debneyi, N. benthamiana and N. clevalandii.* However Biyagama isolate used in this study produced only chlorotic spots in *N.debneyi* and failed to produce any symptoms in *N. claverlendii.* Moreover Biyagama isolate infected two other indicator plants namely *Physalis foetidana* and *Tetragonia expensa.*

Table 1. Host range studies of Cassava Mosaic Virus

Plant Species	*Local reaction*	*Systemic reaction*
Chenapodaceae		
C.amaranticolor	CS	-
C.quinoa	NS	-
C.murale	-	-
C.foetidum	-	-
Solanaceae		
Capsicum annum	-	-
Physalis foetidana	CS	-
Nicotiana benthamiana	NS	MO
N.glutinosa	CS	LD,ST
N.debneyi	CR	-
N.tabacum cv. White Burly	-	-
N.tabacum cv bright yellow	-	LD
N. Xanthi	-	-
N.clavelendii	-	-
N.rustica	-	-
N.tabacum Hawana	CP	M
Cruciferae		
Choisum		
Citrullus vulgaris	-	-
Beta vulgaris	-	-
Euphobaceae		
Manihot esculenta (cuttings)	-	-
Manihot esculenta (seedling)	NS	M
Euphobia heterophila	-	-
Ricinus communis	-	-
Wild castor *(Weta endaru)*	-	-
Aizoaceae *Tetragonia expensa*		
Compositae		
Zinia eleganca	-	-
Leguminoceae		
Cassia occidentalis		
Cucurbitaceae		
Water melon		

Abbreviations- CS – Chlorotic spots, NS – Necrotic spots, CR – Chlorotic rings, MO – Mottling, LD – Leaf distortion
ST - Stunting , M - Mosaic, CP - Chlorotic patches

Table 2. Results of graft transmission

Scion (Diseased)	*Rootstock*	*Reaction*
Cassava	Cassava	Systemic mosaic
Cassava	Castor	No reaction

Cassava mosaic virus can be successfully transmitted to healthy cassava by chip grafting.

Table 3. Physical properties of Cassava Mosaic Virus

Thermal Inactivation Point (TIP)	45-50^{0}C
Dilution end point (DEP)	10^{-2}- 10^{-3}
Longivity *in-vitro* (LIV)	4- 6 d

Physical properties of cassava mosaic virus have shown that it is a moderately stable virus.

Virus Purification and Antiserum Production

Success level of Partially purified antiserum was tested by inoculating diluted pellet to *Nicotiana benthamiana* which produced necrotic spots followed by systemic mottling. Infectivity tests confirmed the presence of the virus in partially purified preparation. However in sucrose density gradient centrifugation it was not possible to get a purified virus zone. Instead a pellet was obtained. Therefore after partial purification, differential centrifugation was done for further purification. Absorbed antiserum was mixed with an equal volume of glycerol and 0.1% Na azide for preservation.

Table 4. ELISA absorbance values obtained from different buffers used for CaMV extraction

Virus Isolate	*.05 M Carbonate buffer pH 9.6 + 10 mM NaDIECA*	*0.1M Phosphate buffer pH 7.8 + .01 M NaEDTA*
Healthy	.017	.0015
Threshold value	.034	.003
Biyagama	.433 +	.261 +
Panagoda	.192 +	.258 +
Habarakada	.225 +	.090 +
Maradana	.345 +	.435 +
Homagama *	.028 (-)	-.004 (-)
Alawwa *	.227 +	.100 +

+ = positive; , (-) = negative, NaDIECA – Sodium di-ethyl dithio carbamic acid
NaEDTA - Sodium ethylene diamine tetra acetic acid * Wild Casava
Absorbance values greater than twice that of healthy were considered as positive for cassava mosaic virus.

Results showed that the carbonate buffer (0.05 M Carbonate buffer, pH 9.6, 10 mM NaDIECA) was better than the phosphate buffer for virus extraction from cassava leaves. Cassava isolates and one wild species of cassava wild rubber sample collected from Homagama gave negative results for both extraction buffers used in the tests. This may be due to a different virus present in the sample. Therefore in routine tests, carbonate buffer was used to extract the virus from cassava leaves. Further it has confirmed that the locally produced antiserum has the capability to detect cassava mosaic virus in cassava leaves.

CONCLUSIONS

The Virus isolated from cassava had a narrow host range. It can be transmitted by both sap inoculation and grafting methods. Physical properties of the virus revealed that cassava mosaic virus is moderately stable. Partial purification was possible, however, it was difficult to purify the virus by sucrose density gradient centrifugation. Polyclonal antiserum effectively detected cassava mosaic virus in cassava when it was detected with .05 M carbonate buffer pH 9.6 + 10 mM Na DIECA by indirect ELISA. Detection method could be utilized to differentiate diseased from healthy cassava plants. This will be useful in future for virus free planting material production programmes.

ACKNOWLEDGEMENTS

Authors thank Dr. (Mrs.) S. Jayasekera and Dr. M.G. Thammitiyagoda of Animal section, Medical Research Institute, Colombo for their kind assistance and facilities provided for carrying out this programme for antiserum production. They are grateful to Dr. (Mrs.) N. Salim of University of Sri Jayawardenapura for exchanging views on the subject.

REFERENCES

Aiton, M.M. and B.D. Harrison. 1989 Monoclonal antibodies to Indian cassava mosaic geminivirus (ICMV). Report of the Scottish Crop Research Institute for 1989: 175 p.

Bock, R, E.J. Guthrieand, G. Meredith. 1978. Distribution, host range, properties and purification of cassava latent virus, a gemini virus. Annals of Applied Biology 90: 361-367.

Costa, A.S. and E.W. Kitajima. 1972. Cassava common mosaic virus CMI/ABB. Descriptions of Plant Viruses No 90. Commonwealth Agricultutal Bureaux, Slough.

Lennon, A.M., M.M. Aiton and B.D. Harrison, 1986. Cassava viruses from South America. 167 p. *in* Annual Report 1985, Scottish Crop Research Institute, Dundee.

Mowat, W.P. and S.Dawson. 1987. Detection and identification of plant viruses by ELISA using crude sap extracts and unfractionated antisera. J. of Virological Methods 15: 233-247

Noordam, D. 1973. Identification of Plant Viruses. Methods and Experiments. 50-56

Pp. Centre for Agricultural Publishing and Documentation, Wageningen.

Saunders Keith, Nazeera Salim, Vasant R. Mali, Varagur G.Malathi, Rob Britton, Peter G. Markham and John Stanley 2002. Characterization of Sri Lankan Cassava Mosaic Virus and Indian Cassava Mosaic Virus. Evidence of acquisition of a DNA component by a Monopartite *Begomovirus*. Virology 293. 63-74 (2002).

Sequiera, J.C. and B.D. Harrison. 1982 Serological studies on cassava latent virus. Annals of Applied Biology. 101: 33-42 Pp.