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Development of recombinant coat protein antibody based IC-RT-PCR for detection and discrimination of sugarcane streak mosaic virus isolates from Southern India*

Brief Report

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Summary. Sugarcane streak mosaic virus (SCSMV), causes mosaic disease of sugarcane and is thought to belong to a new undescribed genus in the family *Potyviridae*. The coat protein (CP) gene from the Andhra Pradesh (AP) isolate of SCSMV (SCSMV-AP) was cloned and expressed in *Escherichia coli*. The recombinant coat protein was used to raise high quality antiserum. The CP antiserum was used to develop an immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) based assay for the detection and discrimination of SCSMV isolates in South India. The sequence of the cloned PCR products encoding 3′untranslated region (UTR) and CP regions of the virus isolates from three different locations in South India viz. Tanuku (Coastal Andhra Pradesh), Coimbatore (Tamil Nadu) and Hospet (Karnataka) was compared with that of SCSMV-AP. The analysis showed that they share 89.4, 89.5 and 90% identity respectively at the nucleotide level. This suggests that the isolates causing mosaic disease of sugarcane in South India are indeed strains of SCSMV. In addition, the sensitivity of the IC-RT-PCR was compared with direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA) and dot-blot immunobinding assays and was found to be more sensitive and hence could be used to detect the presence of virus in sugarcane breeding, germplasm centres and in quarantine programs.

* The nucleotide sequences reported in this paper have been assigned the accession numbers – AY 189681, AY 193783, AY 193784 for SCSMV-TA, SCSMV-KA, SCSMV-TN respectively by the GenBank.

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Sugarcane (*Saccharum officinarum L.*) is an important commercial crop and India ranks as one of the top producers of cane sugar. Sugarcane streak mosaic virus (SCSMV-AP) causes mosaic disease of sugarcane in India [7, 10]. Incidence of this disease is nearly 100% in major sugarcane growing states in India and results in significant loss of yield [1, 13].

SCSMV is a flexuous filamentous virus (890 × 15 nm) with a monopartite ssRNA genome, approximately 10 kb in length [7]. The virus induces pinwheel and laminated aggregate type of inclusions that are characteristic of members in the family *Potyviridae* [8, 18]. It is transmitted through vegetative porpagules (setts) under natural conditions [7, 10]. SCSMV was suggested to be a putative member of the genus *Tritimovirus* in the family *Potyviridae* based on limited homology (30%) in the coat protein (CP) and 3′-untranslated region (UTR) [6, 7]. Recently, molecular cloning and sequencing of SCSMV-AP at the NIb region suggested that it could belong to an undescribed new genus of the family *Potyviridae* [10, 17]. Further, based on biological, serological and molecular characteristics, the virus isolates causing mosaic disease of sugarcane in South Indian states were identified as pathotypes of SCSMV [11].

The quality of the polyclonal anti bodies produced against a virus depends on the purity of virus preparation used for immunization. Though ELISA was found to be useful for routine large-scale detection of the SCSMV-AP, polyclonal antibodies raised against purified virions cross react with host antigens and often give variable background reactions, thus limiting their use in ELISA for diagnosis of virus infection [9]. Therefore, production of antibodies to recombinant CP with the potential of working in all serological tests with no background reaction will be useful in diagnosis and such high quality antibodies would be useful for developing antibody based PCR tests.

For successful implementation of control programs for viruses propagated through vegetative plant parts, it is necessary to have a rapid, reliable, sensitive test to screen germplasm in quarantine and to determine the distribution of the virus in commercial fields. Sometimes symptomless canes may carry virus and may not be detected by serological methods. Detection of SCSMV-AP by nucleic acid hybridization tests gives background with healthy samples due to pigment interference [9]. Attempts to detect SCSMV by RT-PCR has been hampered by technical difficulties in obtaining suitable RNA preparations from sugarcane tissue and the method is not amenable for large scale indexing. Immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) is a sensitive technique that allows for the detection of viruses in oxidising plant extracts and inhibitory plant constituents are washed from immobilized virions. [15, 16, 19, 25]. Partial nucleotide sequences covering 3′-UTR and CP coding region are useful in distinguishing strains of a virus from distinct viruses in the family potyviridae [2, 5, 22]. No sequence information of SCSMV isolates other than that of SCSMV-AP are available from India [7, 11].

In this paper, we describe the production of high quality polyclonal antiserum against recombinant SCSMV-AP CP and the development of an IC-RT-PCR

technique using recombinant CP antiserum for the detection of virus. This is the first report on the application of recombinant CP antibody based IC-RT-PCR for the detection of SCSMV isolates. We show that the technique is more sensitive compared to other serological methods.

Virus isolates used in this study were collected from commercial sugarcane infected with mosaic disease in Tirupati (Chittoor district; Andhra Pradesh) (SCSMV-AP), Tanuku (West Godavari district, Coastal Andhra Pradesh) (SCSMV-TA), Hospet (Bellary district; Karnataka) (SCSMV-KA) and Coimbatore (Tamil Nadu) (SCSMV-TN), India. All isolates were maintained on *Sorghum bicolor* cv. Rio plants grown inside the wiremesh house by periodic sap inoculation and also through sugarcane setts [7, 11].

The CP gene (849 nucleotides) was amplified from cDNA clone (pSCSMV) [7] using the following set of virus specific primers (designed based on N and C terminal SCSMV-AP CP sequence) for cloning into the pRSET-A expression vector.

(i) Sense primer : 5′ TTGGATCCGGACAAGGAACGCAGCCACCTC 3′

(ii) Antisense primer: 5′ TATAAAGCTTTCAGTGCTGAGCACGCCCAAAC TG 3′

The underlined nucleotides represent *Bam*HI and *Hin*dIII sites. The CP gene was amplified using Deep Vent DNA polymerase (New England Biolabs, Inc, MA) under the following conditions: 94 °C, 3 min (1 cycle); 94 °C, 30 sec denaturation; 58 °C, 30 sec annealing and 72 °C, 1 min extension (30 cycles) followed by an extension step at 72 °C, 10 min. The amplified product was gel purified and digested with *Bam*HI and *Hin*dIII and cloned into *Bam*HI/*Hin*dIII digested pRSET-A (Invitrogen) expression vector which resulted in the addition of extra 36 amino acids from vector along with His tag (34 from the pRSET-A vector and 2 from sense primer) at the N-terminus of the CP (pR-SCCP). *E. coli* BL 21 (DE3) pLys S (Stratagene) was used as the expression host.

For expression, one percent of overnight grown culture of *E. coli* BL 21 (DE3) pLys S cells, harboring pR-SCCP was inoculated to 100 ml terrific broth [20] containing 50 μg/ml ampicillin. After 3 h of growth, the cells were induced with 0.5 mM IPTG and harvested after 4 h. The cells were resuspended in 100 mM Tris-HCl buffer (pH 8.0). The cell suspension was sonicated using a Vibra cell sonicator in an icebath and subjected to centrifugation at 12000 rpm for 10 min. The recombinant coat protein from the soluble fraction was purified using Ni-NTA column chromatography (Qiagen Inc, Chatsworth, CA) according to manufacturer's instructions. Fractions were analysed by 12% SDS-PAGE [14] and the protein was estimated by UV-Spectrophotometry. The expression of recombinant CP was confirmed by Western analysis [4] using polyclonal antiserum raised against purified SCSMV-AP (conventional antiserum) [7].

Fractions of Ni-NTA column purified recombinant CP were concentrated by using centrifugal filter device (Novagen) and used as immunogen to produce polyclonal antiserum in a 12 week old New Zealand white rabbit. Pre-immune serum was collected one week before immunization. The purified recombinant CP (300 μg) was emulsified in Freund's complete adjuvant for the first injection and

this was followed by four injections of 150 μg protein in Freund's incomplete adjuvant. The injections were given intramuscularly at 21 day intervals. The serum titres and specificity were determined by direct antigen coating (DAC)-ELISA [12] and western blot analysis [4] using purified recombinant CP, purified virus and leaf antigens.

IC-RT-PCR was performed as described by Nolasco et al. (1993) [16] except that the immunocaptured virions were disrupted at 80 °C for 10 min, chilled on ice. Plant extracts were prepared from young healthy, SCSMV infected sorghum and sugarcane (healthy sugarcane sample obtained from University of Agricultural Sciences, Bangalore, India) leaves by grinding in extraction buffer (500 mM Tris-Cl, pH 8.3, containing 0.01 M Na_2SO_3, 2% Polyvinylpyrrolidone MW 40,000, 3 mM NaN_3, 140 mM NaCl and 0.05% Tween 20) at 1:10 dilution. The extracts were clarified by centrifugation (10000 × g, 10 min). Sugarcane leaf samples collected from Agricultural Research Station (Tirupati, A.P., India) and infected leaf samples from coastal Andhra Pradesh, Tamil Nadu and Karnataka were also processed as described above. Recombinant coat protein antiserum was used at 1:1000 dilution for capturing of virions from crude extracts. For IC-RT-PCR, 3′ antisense primer corresponding to the 3′ terminal 30 nucleotides in SCSMV-AP sequence (5′TTTTTTCCTCCTCACGGGGCAGGTTGATTG 3′) and sense primer corresponding to the N-terminal 8 amino acids of SCSMV-AP CP sequence (5′GGA CAA GGA ACG CAG CCA CCT CAG 3′) were used and PCR was done using the conditions as described above for the CP amplification. The products were analyzed by 1% agarose gel electrophoresis in TAE buffer [20].

IC-RT-PCR products (expected size of 1.1 kb encoding entire CP and 3′-UTR were excised from the gel and eluted using GENE CLEAN Kit (Novagen) and the DNA fragments were cloned directly into PGEM-T Easy vector (Pharmacia-Amersham Biotech). For sequencing, plasmids with inserts of the expected size were grown in *E. coli* DH5 α cells and purified by alkaline lysis method [20]. At least two independent clones from each isolate were sequenced by Sanger's dideoxy chain termination method [21] on ABI prism automated DNA sequenator. Nucleotide and amino acid sequences were analysed and compared using the Genetics computer Group (GCG) sequence analysis software from the University of Wisconsin and Clustal W [23].

The direct antigen coating version of ELISA (DAC-ELISA) [12] and dot-blot immunobinding assay [3] were performed with the sugarcane leaf samples collected from Agricultural Research Station (Tirupati, A.P., India) along with infected and healthy controls. Microtitre plates (Nunc, Maxisorp) for DAC-ELISA and nitrocellulose strips (Ambala Cantt) for dot-blot immunobinding assay were coated with leaf extracts (100 μl and 5 μl respectively) prepared in coating buffer, pH 9.6 (1 g/9 ml). Recombinant CP antiserum (1:1000) was used as primary antibody and AP/HRP labelled-goat antirabbit antibodies (DAC-ELISA and dot-blot immunobinding assay) (Genei, India) were used as secondary antibody. Substrates used were disodium p-nitrophenyl phosphate in 10% diethonolamine for AP and DAB/H_2O_2 (Sigma) for HRP.

PCR amplified SCSMV-AP CP (283 amino acids) was expressed in *E. coli* BL 21 (DE3) pLys S cells. Nearly 90% of the expressed protein was in the soluble fraction and the recombinant CP was purified from this fraction by Ni-NTA affinity chromatography (Fig. 1A, lane 4). The authenticity of the expressed protein was confirmed by western blot analysis using conventional antiserum (Fig. 1B, lanes 2, 3) and no band was observed from the protein extracted from the uninduced cells (Fig. 1A, lane 2; Fig. 1B, lane 1). The virus purified from infected leaves was partially degraded (Fig. 1A lane 1 and Fig. 1B lane 4).

Recombinant CP was used as an immunogen to produce polyclonal antiserum as described in the methods section. The specificity of the recombinant CP based antiserum was determined by western blot analysis using purified virus, purified recombinant CP and leaf extracts and the titres were determined by DAC-ELISA. The recombinant CP antiserum at 1:10000 dilution could detect SCSMV in infected sugarcane and sorghum leaf extracts and was virtually free of background reaction (data not shown). Conventional antiserum produced against SCSMV-AP gave background at lower dilutions in serological tests [9]. Thus the recombinant CP based antiserum is very useful for routine diagnosis and CP can be used as a non-infectious positive control as it poses no quarantine problems. The clone can be stored indefinitely, cultured when required, CP overexpressed and purified in good yield.

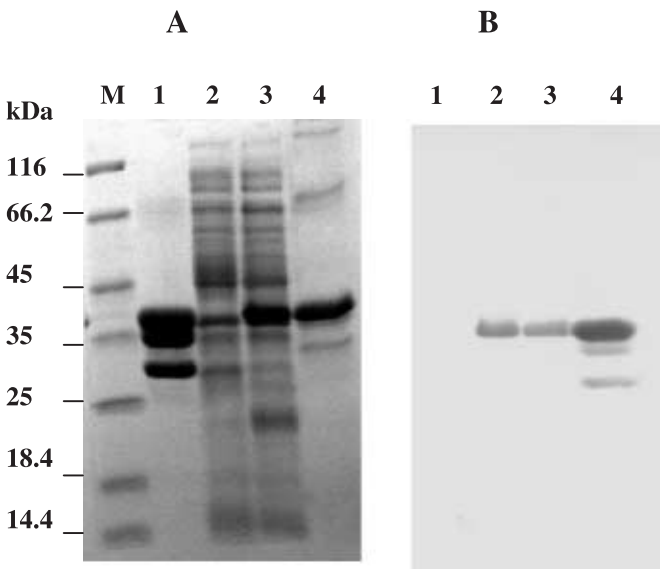

Fig. 1. Electrophoretic analysis of expressed recombinant CP in *E. coli*. The BL 21 (DE3) pLys S *E. coli* cells harboring pR-SCCP were induced with 0.5 mM IPTG. The expressed protein was analyzed by SDS-PAGE (**A**) *M* molecular weight markers; *1* disrupted purified virus (SCSMV-AP); *2* total protein from uninduced cells; *3* from induced cells; *4* purified recombinant CP and western blotting (**B**) using SCSMV-AP conventional antiserum. *1* total protein from uninduced cells; *2* from induced cells; *3* purified recombinant CP; *4* disrupted purified virus (SCSMV-AP)

The technique of capturing SCSMV from diseased tissue sample in microfuge tubes coated with recombinant CP antibodies and amplifying the viral sequences by RT-PCR in the same tube, promoted the detection of SCSMV-AP in sorghum and sugarcane infected leaf extracts with high sensitivity and specificity. Using this approach, the isolates collected from different locations of South Indian states such as SCSMV-TA, SCSMV-TN, SCSMV-KA were also subjected to IC-RT-PCR and the PCR products of expected size were obtained. The IC-RT-PCR products were cloned and sequenced as described in the methods section. The sequences were more homologous in the 3′ UTR and C terminal half of the CP than in the N terminal half. The SCSMV-TA (AY 189681), TN (AY 193784) and KA (AY 193783) isolates showed 98–99% identity at the nucleotide level with each other and 89.4, 89.5 and 90% identity with SCSMV-AP (Y 17738), respectively. However, since all of them showed an overall percent identity $> 85\%$ they can all be considered as strains of SCSMV [24]. In India, though several strains of sugarcane mosaic potyvirus were reported to exist based on differences in physical properties, host range, particle morphology and limited serological data, none of them have been characterized at the molecular level [7]. The results presented

Table 1. Comparative analysis of DAC-ELISA, dot-blot immunobinding assay and IC-RT-PCR for the detection of SCSMV

S.no	Fig. 2 lane no.	Cultivar/variety	Type of the symptom	DAC-ELISA	DBIA	IC-RT-PCR
1	4	83 V 15	MM	(0.24) −	+	+
2	5	93 V 297	SM	(2.10) +	+	+
3	6	89 V 74	MM	(0.38) −	+	+
4	7	CO V 94101	AS	(0.18) −	−	+
5	8	85 A 261	MoM	(0.98) +	+	+
6	9	93 A 10	AS	(0.16) −	−	+
7	10	93 R 113	MM	(0.16) −	−	+
8	11	85 R 196	MoM	(1.05) +	+	+
9	12	COC 99065	MM	(0.19) −	−	+
10	13	CO 7805	SM	(1.98) +	+	+
11	14	C 960696	AS	(0.13) −	−	+
12	3	Healthy sugarcane	AS	(0.14) −	−	−

'+' samples indexed positive; '−' samples indexed negative

Numbers in parenthesis represent the values of average absorbance of A_{405} of three wells recorded after 90 min of adding the substrate and readings thrice to that of healthy sugarcane are considered as positive

The samples were analysed in parallel as described in materials and methods from symptomatic plants which included mild mosaic (*MM*), moderate mosaic (*MoM*), severe mosaic (*SM*) and asymtomatic plants (*AS*) of representative popular sugarcane cultivars. Healthy sugarcane obtained from University of Agricultural Sciences, Bangalore, India was included along with the samples. The cultivars are designated as follows. First number represents the year of release and next letter represents the place of research station (*V* – Vuyyur (Krishna dt, A.P); *CO* – Coimbatore (Tamil Nadu); *C* – Cuddalore (Tamil Nadu); *A* – Anakapalle (Vishakapatnam dt, A.P); Rudrur (Nizamabad dt, A.P) that developed the cultivar and last number represents the serial number

here show that the mosaic disease of sugarcane in India is due to different strains of SCSMV.

To test the sensitivity of IC-RT-PCR in routine detection of SCSMV, a comparative analysis was carried out with three detection methods viz. DAC-ELISA, dot immunobinding assay and IC-RT-PCR using representative popular sugarcane cultivars collected from Agricultural Research Station, Tirupati, A.P, India. A range of leaf samples with mild to severe symptoms along with samples from asymptomatic canes were tested at a single dilution in all the three tests (Table 1). The results indicated that all the samples that were positive by ELISA (4/11) were also positive by dot-blot immunobinding assay and IC-RT-PCR. Using dot-blot immunobinding assay, virus could be detected in two additional samples (6/11), confirming its higher sensitivity when compared to DAC-ELISA test (Table 1). Using IC-RT-PCR, the virus could be detected in (8 out of 8) symptomatic samples (Table 1; Fig. 2, lanes 4–6, 8 and 10–13), and (3/3) asymptomatic samples (Table 1; Fig. 2, lanes 7, 9 and 14). The band observed from one sample with mild mosaic symptoms (83 V 15, lane 4) and one sample with asymptomatic symptoms 93 A 10 (Fig. 2, lanes 9) was rather weak and although visible, could not be reproduced upon photography. The results demonstrate the higher sensitivity of

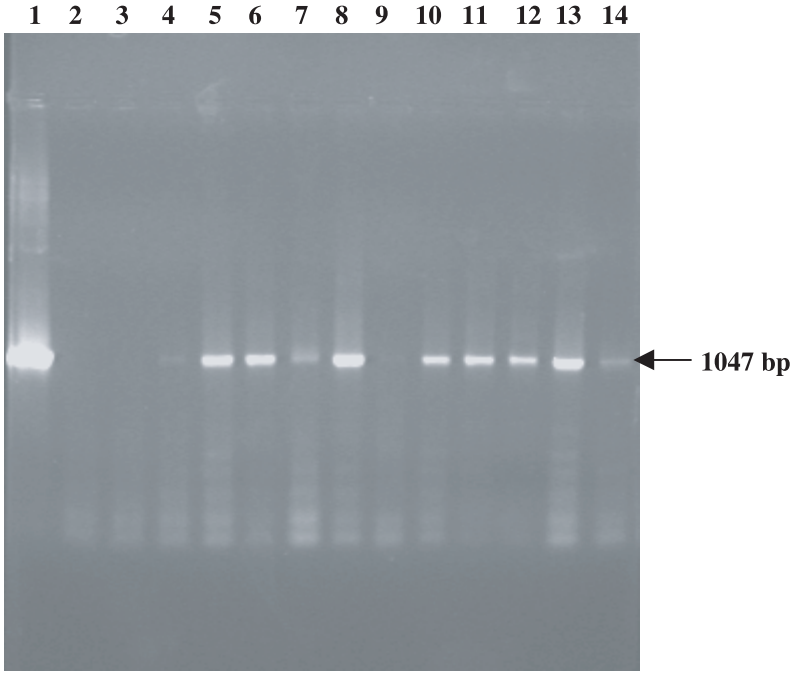

Fig. 2. Detection of SCSMV in representative popular sugarcane cultivars by IC-RT-PCR. Analysis of sugarcane leaf samples was carried as described in methods section. *1* PCR product from pSCSMV-AP cDNA clone (positive control); *2* template (negative control); *3* healthy sugarcane (obtained from University of Agricultural Sciences, Bangalore, India); *4–14* corresponds to cultivars 83 V 15, 93 V 297, 89 V 74, COV 94101, 85 A 261, 93 A 10, 93 R 113, 85 R 196, COC 99065, CO 7805, C 960696, respectively

IC-RT-PCR when compared to DAC-ELISA and dot-blot immunobinding assay. Though ELISA can be used for routine large scale testing of virus, sensitivity is the limitation and in dot-blot immunobinding assay pigments may interfere and give background reaction. Thus the recombinant CP antibody based IC-RT-PCR described here can be used in diagnosis as well as in strain discrimination, particularly of viruses which are difficult to purify and in samples with no obvious symptoms. This method can be applicable for the detection of virus in planting material needed for virus elimination programs and also in screening programs at sugarcane germplasm and breeding centres.

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References

1. Agnihothri VP (1996) Current sugarcane disease scenario and management strategies. Ind J Phytopathol 49: 109–126
2. Atreya CD (1992) Application of genome sequence information in potyvirus taxonomy: an overview. In: Barnett OW (ed), Potyvirus taxonomy. Springer, Wien New York. Arch Virol [Suppl] 5: 17–23
3. Berger PH, Thornbury DW, Pirone TP (1985) Detection of picogram quantities of potyviruses using dot-blot immunobinding assay. J Virol Methods 12: 31–36
4. Burgermeister W, Koenig R (1984) Electroblot immunoassay – A means for studying serological relationships among plant viruses. Phytopathol Z 111: 15–25
5. Frenkel MJ, Ward CW, Shukla DD (1989) The use of 3′ non coding sequence in the taxonomy of potyviruses: application to watermelon mosaic virus 2 and soybean mosaic virus. J Gen Virol 70: 2775–2783
6. Hall JS, Adams B, Parsons TJ, French R, Lane LC, Jensen SG (1998) Molecular cloning, sequencing and phylogenetic relationships of a new potyvirus: sugarcane streak mosaic virus and a reevaluation of the classification of the *Potyviridae*. Mol Phylogen Evol 10: 323–332
7. Hema M, Joseph J, Gopinath K, Sreenivasulu P, Savithri HS (1999a) Molecular characterization and interviral relationships of a flexuous filamentous virus causing mosaic disease of sugarcane (*Saccharum officinarum L.*) in India. Arch Virol 144: 479–490
8. Hema M, Savithri HS, Sreenivasulu P (2001a) Sugarcane streak mosaic virus: occurrence, purification characterization and detection. In: Rao GP, Ford RE, Tosic M, Teakle DS (eds), Sugarcane pathology, vol. 2, Virus and phytoplasma diseases. Science Publishers, Inc, USA, pp 37–70

9. Hema M, Savithri HS, Sreenivasulu P (2001b) Antibody and nucleic acid probe-based techniques for detection of sugarcane streak mosaic virus causing mosaic disease of sugarcane in India. Curr Sci 81: 1105–1108
10. Hema M, Sreenivasulu P, Savithri HS (2002) Taxonomic position of sugarcane streak mosaic virus in the family *Potyviridae*. Arch Virol 147: 1997–2007
11. Hema M, Venkatramana M, Savithri HS, Sreenivasulu P (1999b) Biological, antigenic and genomic relationships among the virus isolates causing mosaic disease of sugarcane in south India. Curr Sci 77: 698–702
12. Hobbs HA, Reddy DVR, Rajeshwari R, Reddy AS (1987) Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. Plant Dis 71: 747–749
13. Jain RK, Rao GP, Varma A (1998) Present status of management of sugarcane mosaic virus. In: Hadidi A, Khetarpal RK, Koganezava H (eds), Plant virus disease control. American Publishing Company, Minnesota, pp. 495–523
14. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 680–685
15. Mumford RA, Seal SE (1997) Rapid single tube immunocapture RT-PCR for the detection of two yam potyviruses. J Virol Methods 69: 73–79
16. Nalasco G, de Blas C, Torres V, Ponz F (1993) A method combining immunocapture and PCR amplification in a microtiter plate for the detection of plant viruses and subviral pathogens. J Virol Methods 45: 201–218
17. Rabenstein F, Seifers DL, Schubert J, French R, Stenger DC (2002) Phylogenetic relationships, strain diversity and biogeography of tritimoviruses. J Gen Virol 83: 895–906
18. Riechmann JL, Lain S, Garcia JA (1992) Highlights and prospects of potyvirus molecular biology. J Gen Virol 73: 1–16
19. Rowhani A, Maningas MA, Lile LS, Daubert SD, Golino DA (1995) Development of a detection system for viruses of woody plants based on PCR analysis of immobilized virions. Phytopathology 85: 347–352
20. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York
21. Sanger F, Nicklen S, Caulson AR (1977) DNA sequencing with chain termination inhibitors. Proc Natl Acad Sci USA 74: 5463–5467
22. Shukla DD, Ward CW (1988) Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. J Gen Virol 69: 2703–2710
23. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680
24. Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens E, Esters E, Lemon S, Maniloff J, Mayo MA, McGeoch D, Pringle, CR, Wickner RB (2000) Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, New York, pp. 1162
25. Wetzel T, Candresse T, Macquaire G, Ravelonandro M, Dunez J (1992) A highly sensitive immunocapture polymerase chain reaction method for plum pox potyvirus detection. J Virol Methods 39: 27–37

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