Research Article (Open access)

Serological Detection of Cardamom Mosaic Virus Infecting Small Cardamom, *Elettariacardamomum* L.

Snigdha Tiwari [1*], Anitha Peter[2], Shamprasad Phadnis[3]

[1]Ph. D Scholar, Department of Molecular Biology and Genetic Engineering, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarkhand, India

[2]Associate Professor, Department of Plant Biotechnology, UAS (B), GKVK, Bangalore, India

[3]Ph.D. Scholar, Department of Plant Biotechnology, UAS (B), GKVK, Bangalore, India

[*]**Address for Correspondence:** Snigdha Tiwari, Ph. D Scholar, Department of Molecular Biology and Genetic Engineering, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarkhand, India

Received: 20 April 2016/Revised: 19 May 2016/Accepted: 16 June 2016

ABSTRACT- Small Cardamom (*Elettariacardamomum* L. Maton) is one of the major spice crops of India, which were the world's largest producer and exporter of cardamom till 1980. There has however been a reduction in production, mainly because of Katte disease, caused by cardamom mosaic virus (CdMV) a potyvirus. Viral diseases can be managed effectively by early diagnosis using serological methods. In the present investigation, CdMV isolates were sampled from Mudigere, Karnataka, ultra purified, and electron micro graphed for confirmation. Polyclonal antibodies were raised against the virus and a direct antigen coating plate Enzyme linked immunosorbent Assay (DAC-ELISA) and Dot-ELISA (DIBA) standardized to detect the virus in diseased and tissue cultured plants. Early diagnosis in planting material will aid in using disease free material for better yields and hence increased profit to the farmer.

Key-Words: Cardamom mosaic virus (CdMV), Electron microscopy, Direct antigen coating Enzyme linked immunosorbent Assay (DAC-ELISA), Dot-ELISA (DIBA)

----------IJLSSR----------

INTRODUCTION

Small cardamom (*Elettariacardamomum* L. Maton), popularly known as 'Queen of spices', is one of the major spice crops and enjoys a unique position in the international spice market. It is one of the highly prized spices of the world and is the third most expensive spice after saffron and vanilla. It belongs to the family Zingiberaceae and is indigenous to southern India [1]. India was the world's largest producer and exporter of cardamom till the 1980s but, by 1990s Guatemala emerged as the leading producer and exporter of cardamom [2]. The production area of cardamom in India has drastically come down from 1.05 lakh ha (1987-88) to only 71, 110 ha (2011-12) (www.mcxindia.org).

Access this article online	
Quick Response Code:	Website: www.ijlssr.com
ISSN 2455-1716	DOI: 10.21276/ijlssr.2016.2.4.5

Viral diseases of cardamom is one of the major reason for low productivity of cardamom (145 kg/ha) [3-4]. 'Katte' or Mosaic or Marble (Annamalai) disease being one of the most destructive among them [5]. The disease is caused by *Cardamom mosaic virus* (CdMV), belonging to the genus *Macluravirus* of family *Potyviridae*which appears as flexuous filamentous particles of about 650 nm in length and 10-12 nm in diameter [4]. In diseased plants, the morphological characteristics include interrupted pale green stripes which run along the veins and parallel to each other from midrib to margin of the leaf. In advanced stages the pale green stripes are distributed evenly over the leaf surface giving a distinct mosaic pattern. The disease incidence was found to be 0.01 to 99.00 per cent in cardamom growing tracts in South India [6]. The virus is transmitted through the banana aphid, *Pentalonia nigronervosa* and infected rhizomes [7]. The virus exists as a symptomless carrier or with mild symptoms in the planting material [8].

Management of 'katte' is difficult as there is no vector control, alternate control or appropriate cultural practices. Disease resistant cardamom varieties released against Katte disease are met with little success, as they showed poor agronomic character. Breeding for resistance and cross

protection are the traditional methods of plant virus control [9] which shows only limited success in this crop as there isn't a single source of viable resistance in the species of *Elettaria*. Other related genera which are infected by the same virus are *Ammomum, Alpinia, Curcuma*, etc. Incompatibility barriers have prevented the formation of fruits in intergeneric hybridization [2]. Though tissue culture techniques have been attempted for producing virus free cardamom plantlets, it is unsuccessful [10].

In this scenario, one of the promising methods of managing the disease is through early diagnosis using serological techniques. This paper reports the purification and electron microscopy of the virus and the standardization of a plate Enzyme Linked Immunosorbent Assay (ELISA) and a Dot-ELISA (DIBA) for the detection of the virus.

MATERIALS AND METHODS

Virus isolation, purification and Electron microscopy

Cardamom plants infected with CdMV var. Mudigere-1 and Mudigere-2 showing characteristic symptoms of cardamom mosaic: alternate light yellow patches and dark green patches with a characteristic mosaic pattern on upper surface of leaf (Fig.1) were collected from Zonal Agriculture Research Station, Mudigere, Karnataka, 2008. The experiments were conducted in dept. of Biotechnology, UAS, Bangalore, IVRI, Bangalore and NIMHANS, Bangalore, 2008.

The virus was extracted by modification of procedure given by [11]. Infected leaves were ground in liquid nitrogen and homogenized with two and-a-half volumes (1g/2.5ml) of 0.1 M potassium phosphate buffer, pH 8.0 containing 0.1 % 2-mercaptaethanol and 0.225 per cent sodium diethyl diethiocarbamate (DIECA). The extract was filtered through double layered muslin cloth. Ten per cent cold chloroform (v/v) was added to the filtrate and emulsified for 15 min. The emulsion was broken by centrifugation at 10,000 rpm for 30 min. The upper aqueous phase containing virus was collected and precipitated by adding six per cent polyethylene glycol (PEG, molecular weight 6000) and 0.2 M sodium chloride. After stirring for 45 min. in ice bath, the preparation was kept in refrigerator overnight. The suspension was centrifuged at 10,000 rpm for 30 min. and precipitate was resuspended in 0.05 M borate phosphate buffer, pH 8.3, containing 0.2 M urea (BPU) and was stirred for 90 min. The suspension was vortexed and centrifuged at 10,000 rpm for 30 min. The supernatant was collected with the help of Pasteur pipette and centrifuged at 24,500 rpm (60,000 g) for 2 h in SW 28 rotor. The supernatant was discarded and the liquid was drained by keeping the tubes in an inverted position on a filter paper. The pellet was resuspended in minimum volume of BPU buffer and centrifuged at 10,000 rpm for 15 min. The supernatant was collected and made upto a required volume by using BPU buffer and centrifuged at 30,000 rpm (1, 10, 000 g) for 2 h. The supernatant was discarded and the liquid was drained off by keeping the centrifuge tubes in an inverted position. The pellet was resuspended in minimum volume of BPU buffer and is layered on 20% sucrose cushion pad and centrifuged at 24,500 rpm for 2 h. Virus pellet was resuspended in 1 ml of 0.05 potassium phosphate buffer, pH 7.0 and centrifuged at 5,000 rpm for 10 min. The supernatant containing virus was collected. The virus suspension was diluted with 0.05 M potassium phosphate buffer. The purified virus suspension was used for electron microscopy.

A drop of purified virus preparation was placed on the carbon-coated grids and allowed to settle for 2-3 min. Excess sample was removed and a small droplet of dye (uranyl acetate) was placed on it for 2-3 min. Excess stain was drained after 15-30 min. and the grids were placed in desiccator and examined under Biotechnai G-2, Transmission electron microscope (TEM) at NIMHANS, Bangalore.

Antiserum Production

New Zealand white rabbit was injected intramuscularly with five 0.5 ml virus injections at weekly intervals. The first was with equal volume of Freund's complete adjuvant and subsequent ones with Fruend's incomplete adjuvant. Test bleed was after the fourth injection, after a ten day gap the final injection was given and final bleeding done ten days later. The serum was separated after overnight incubation at room temperature and antiserum collected [12].

Enzyme linked immunosorbent assay (ELISA)

Indirect plate ELISA was standardized, using different dilutions of infected plant sap (ips) or ultrapurified virus (uv) as antigen, primary antibody was the raised antiserum and secondary antibody was an alkaline phosphatase conjugate. 100 µl of suitably diluted virus in coating buffer was added to the wells of the polystyrene plate, covered and incubated at 4^0C overnight. The plate was then soaked and washed thrice in PBS-T for 3 min. 200 µl of blocking solution was added to the each well of the plate and incubated followed by 100 µl each of suitably diluted primary antibody and secondary antibody alkaline phosphatase conjugate (Goat antirabbitIgG) in blocking buffer. The incubation temperature and duration for each step was 37^0C for 1½ h. The incubation temperature for each step was 37^0C for 1½ h. Wash procedure was repeated after each step. Finally 100 µl of enzyme substrate (para nitro phenyl phosphate) was added and incubated at 37^0C for ½ h. Reaction was terminated with 50 µl of 5N KOH after color development. Visual observation and absorbance values using ELISA reader was taken at 405 nm**.**

The first protocol standardized for ELISA involved the non-addition of PVP and ovalbumin in the blocking buffer (PBS-T) containing 3% fat free milk (ELISA1) and the second with PVP and ovalbumin in the blocking buffer (ELISA2) [13]. The details of the dilutions of the antigen, primary and secondary antibody for ELISA1 and ELISA2 are given in Table 1 and 2 respectively.

DIBA

Dot-Blot was done on nitrocellulose membrane which was dipped in boiling water and equilibrated in PBS. Two microlitre of 1:10, 1:100 and 1:500 dilution of ultrapurified virus was loaded on the membrane using the impression of wells from a manifold with a control, which was the buffer. The dots were deep dried using a vacuum pump for 30 min. The membrane was slowly removed and washed thrice with PBS-T (0.05% TWEEN-20 in PBS) and air-dried. It was then blocked with the blocking solution as above and dotted with different dilutions of primary antibody and followed by a wash step and then coating with the secondary antibody (Table 3). Incubation for each step was at 37˚C for 1½ h. Wash step was repeated after each step (three washes, 3 min. per wash). The membrane was then treated with the substrate bromocresoindoyl pyrophosphate (BCIP) for 10 min in dark to develop color. The primary antibody dilutions used were 1:500 and 1:1000 while that of secondary antibody was 1:1000.

Disease diagnosis

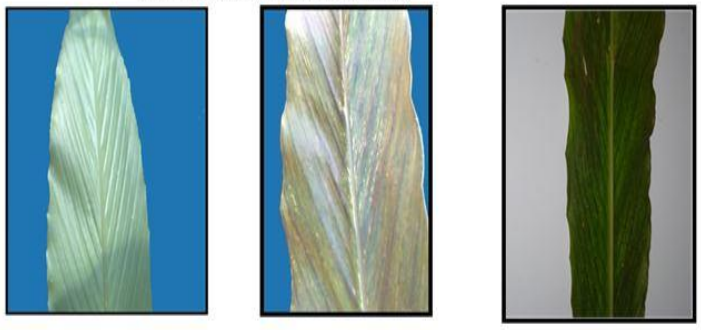
Disease diagnosis was done in infected, healthy and procured tissue cultured plants.

RESULTS AND DISCUSSION

Virus Purification and Electron microscopic study

The virus collected from diseased plants with typical mosaic symptoms (Fig. 1) was subjected to purification protocol described by Srinivasulu *et al.* [11] for peanut mottle virus with modifications, namely using potassium phosphate buffer (pH 8.0) containing 0.225% DIECA, 0.1%, 2-mercaptaethanol and PEG precipitation followed by differential ultracentrifugation and 20 per cent sucrose cushion pad. This method was found to be successful to isolate the virus. The concentration of the virus however was low in the present purification, which may be attributed to the presence of fibrous matrix of the host tissue which interferes with the various stages of purification of the virus which is one of the major drawbacks encountered in many potyvirus purification procedures [14], who has also reported aggregation of virus particles at early stages of purification of CdMV. Similar problems in purification of potyviruses has been reported in Lettuce Mosaic Virus [15], Soybean Mosaic Virus [16], Potato Virus Y[17], Tobacco Etch Virus [18], Pea Seed-borne Mosaic Virus [18], Maize Dwarf Virus [20], and Poplar Mosaic Virus [21]. To avoid this, detergents such as Triton X-100 was used which disrupt the particles of elongated viruses that tend to aggregate and bind to plant organelles. Aggregation on storage is also a deterrent in the purification of many filamentous viruses like CdMV [5,9,10,14], Tobacco Mosaic Virus [22] and Potato Virus Y [23]. The use of urea in extraction and resuspension buffer can also reduce aggregation considerably [9,10,18]. 20% sucrose gradient was used in the present investigation, which is a modification over the original protocol of [11] and [10] to preserve the integrity of the virus particles as reported by earlier workers [24,25].

Fig 1: Cardamom leaves infected with CdMV showing characteristic mosaic symptoms.



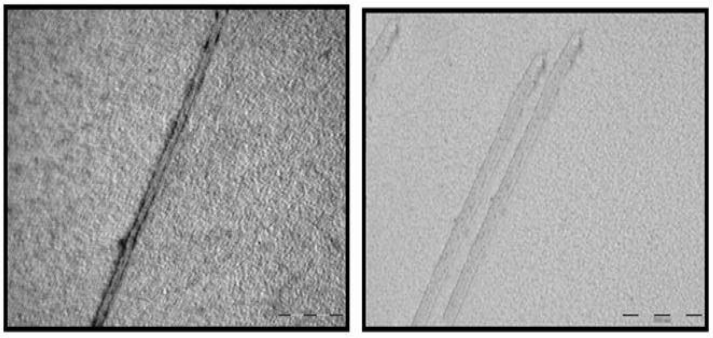
A) Healthy Cardamom plants

B) Mudigeri 1 isolate showing characteristic CdMV mosaic symptoms

C) Mudigeri 2 isolate showing characteristic CdMV mosaic symptoms

Electron microscopy studies confirmed the presence of flexuous rod shaped viral like particles at different magnifications. The size of the virus particles measured was 700nm in length and 12-13 nm in width (Plate 1). The electron microscopy of the purified preparation of CdMV by using two per cent uranyl acetate revealed aggregated numerous flexuous rod shaped particles intertwined together which made it difficult to measure. However average length of 700 nm was obtained among the measured particles. This partial size closely resembled the observation of 700-720 nm [10], 720 nm [14], 710 nm [9].

Plate 1 : Electron micrographs of CdMV showing flexuous rods 750 X 12-13 nm (X 200).



2a. CdMV Virus particle of 700nm in length

2b. Particle of CdMV showing protein frame work of the virus

Antibody production and serological tests

Polyclonal antibodies (PAbs) were successfully raised in rabbit against CdMV by giving 2 intramuscular and subcutaneous injections with the purified virus. Successful production of PAbs against CdMV in rabbits and mice respectively although with a different injection schedule have also been reported [9,10].

Standardization of antibody titer raised against CdMV virion for ELISA

Standardization of antibody titer raised against CdMV virus for ELISA was done to know the suitable concentration of virus and primary antibody for testing the virus. Titre fixation using different dilutions of antiserum with the different concentration of antigen was done in ELISA plates in two runs namely ELISA 1 and ELISA 2 as mentioned earlier.

ELISA 1

The antiserum dilutions used were 1:500, 1:1000, and 1:2000. The concentrations of antigen were undiluted, 1:10, 1:50, 1:100, 1:200, 1:500 and 1:1000. Secondary Antibody dilutions were 1:1000 and 1:2000. In all combinations colour development was observed. Optimum OD reading of 0.886 was observed in the combination of 1000 ng of antigen and 1:500 dilutions of primary antisera with 1:2000 dilutions of secondary antibodies (Table 1).

Table 1: ELISA 1 readings (OD) at 405 nm for the standardization of antibody titre raised against CdMV

		Antigen Dilution							
Primary Antibody Dilution		**1000 ng**	**500ng**	**200ng**	**100ng**	**50 ng**	**10 ng**	**+ve**	**-ve**
		Secondary Antibody Dilution of 1:1000							
	1:500	0.895	0.789	0.632	0.589	0.421	0.190	1.460	0.076
	1:1000	0.824	0.579	0.522	0.395	0.276	0.176	1.207	0.067
	1:2000	0.657	0.498	0.426	0.350	0.210	0.123	1.060	0.087
		Secondary Antibody Dilution of 1:2000							
	1:500	0.886	0.635	0.512	0.492	0.376	0.312	1.346	0.082
	1:1000	0.762	0.642	0.502	0.448	0.346	0.293	1.207	0.067
	1:2000	0.683	0.539	0.483	0.384	0.306	0.216	1.060	0.087

ELISA 2 with PVP and Ovalbumin

The antiserum dilutions used were 1:500, 1:1000, and 1:2000. The concentrations of antigen were undiluted, 1:10, 1:50, 1:100, 1:200, 1:500 and 1:1000, with secondary antibody dilutions of 1:1000 and 1:2000. In all combinations colour development was observed. Optimum OD reading of 0.960 was observed in the combination of 1000 ng of antigen and 1:500 diluted primary antisera with 1:2000 dilutions of secondary antibodies. This combination of 1:100 of antigen and 1:500 dilution of primary antiserum and 1:2000 of secondary antibody was used in further studies (Table 2).

Table 2: ELISA 2 readings (OD) at 405 nm for the standardization of antibody titre raised against CdMV

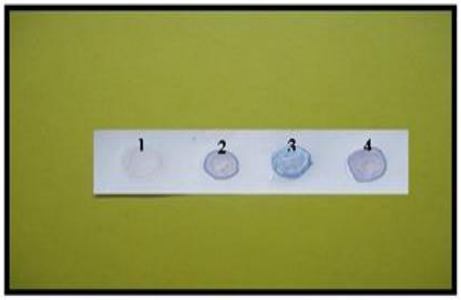
		Antigen Dilution							
Primary Antibody Dilution		**1000 ng**	**500ng**	**200ng**	**100ng**	**50 ng**	**10 ng**	**+ve**	**-ve**
		Secondary Antibody Dilution of 1:1000							
	1:500	1.004	0.894	0.835	0.711	0.624	0.514	1.756	0.078
	1:1000	0.947	0.635	0.487	0.437	0.349	0.351	1.558	0.052
	1:2000	0.828	0.459	0.363	0.339	0.310	0.296	1.410	0.032
		Secondary Antibody Dilution of 1:2000							
	1:500	0.960	0.600	0.432	0.419	0.384	0.331	1.329	0.089
	1:1000	0.629	0.477	0.411	0.289	0.276	0.270	1.218	0.056
	1:2000	0.624	0.391	0.299	0.250	0.235	0.183	1.254	0.067

Standardization of an indirect-ELISA for detecting CdMV at 5µg/ml concentration of primary antibody and 1:1000 and 1:2000 dilution of secondary antibody, and a direct-ELISA at same concentration of primary antibody and 1:400 and 1:800 dilution of secondary antibody [10]. It has been reported that CdMV could be detected at 1:1000 dilution of antigen (leaf extract) and 1:6000 dilution of primary antibody but optimum was 1:100 dilution of antigen and 1:2000 of primary antibody [9]. The results of the present investigation are in accordance with these reports. The ELISA results also revealed that the use of PVP and OA in blocking buffer enhanced the efficiency of ELISA. This is in accordance with the standardized protocols for serological detection of plant viruses [13] and also the protocol used for a related virus, viz the pepper vein banding mosaic virus [26].

Standardization of antibody titre raised against CdMV virus for Dot ELISA

Standardization of antibody titre raised against CdMV virus for ELISA was done to know the suitable concentration of virus and primary antibody raised in rabbit. Titre fixation using different dilutions of antiserum with the different concentration of antigen was done in nitrocellulose membrane. The antiserum dilutions used were 1:500, 1:1000 and 1:2000 with secondary antibody concentration of 1:2000 and antigen concentration of 2 µg/ml. In all combinations colour development was observed in all dilutions except in negative control (containing only coating buffer) (Plate 2). Optimum colour development observed was in 1:1000 diluted antisera. This combination of 1:1000 dilution of antiserum was used in further studies (Table 3).

Plate 2: DOT ELISA for the standardization of antibody titer against CdMV



Dot 1-control without virus
Dot 2- 1:500 antibody dilution
Dot 3- 1: 1000 antibody dilution
Dot 4-1:2000 antibody dilution
With secondary antibody dilution of 1:1000

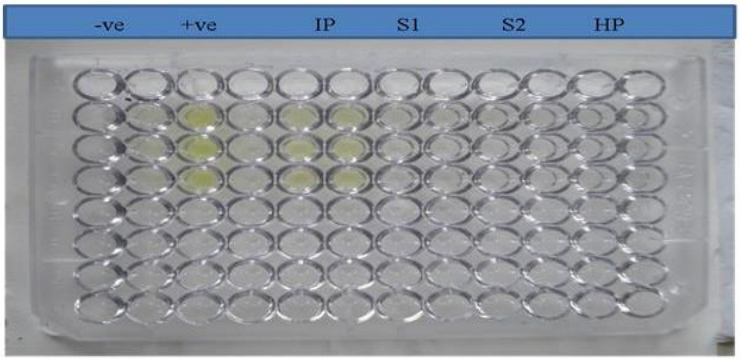
The colour development in all the dots helped in fixation of antibody titre against CdMV. The titre was also confirmed using Dot-ELISA.

Disease diagnosis

The virus was detected in infected plants and not in healthy and procured tissue cultured plants by using standardized ELISA (Plate 3, Table 4 and Figure 2). The virus was detected in infected plants collected from the field and not in healthy plants and the procured tissue cultured plants using ELISA. This can be compared with the findings of direct [10] and indirect ELISA and DOT-ELISA [9] standardized for detection of CdMV in infected plants.

Detection of virus in plants is very difficult and hence attempts have been made to understand the virus and its serological detection by using ELISA for early infection. Early diagnosis in mother plants through conventional multiplication (rhizomes) and micropropagation is required to curtail crop loss and avoid inoculum build and spread.

Plate 3: Detection of CdMV in tissue culture plants by ELISA



-ve Control : Only coating buffer
+ve Control : Ultra Purified Virus
Infected Plants (IP) : Cardamom Plants with CdMV disease
S1: Tissue Cultured Cardamom Plants 1
S 2: Tissue Cultured Cardamom Plants 2
HP: Healthy cardamom plants

Figure 2: ELISA for detection of CdMV in tissue culture plants

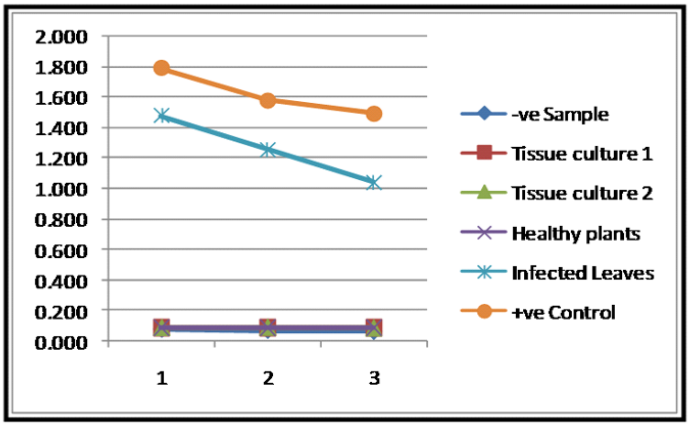


Table 3: Visual scoresof Dot-Blot for Standardization of antibody titre raised against CdMV

Antiserum dilutions	Control	1:500	1:1000	1:2000
Scores	-	++++	++++	+++

++++= Strongly positive, += weakly positive, -=Negative

Secondary antibody: 1:1000 dilution

Table 4: ELISA reading (OD) at 405 for detection of CdMV in tissue culture plants

			Sample			
Antibody	**-ve**	**+ve**	**Infected leaves**	**Tissue culture 1**	**Tissue culture 2**	**Healthy**
1:500	0.0795	1.789	1.480	0.0889	0.0867	0.0876
1:1000	0.0724	1.579	1.257	0.0860	0.0880	0.0879
1:2000	0.0657	1.498	1.043	0.0840	0.0838	0.0836

Secondary antibody of 1:1000 dilution

CONCLUSIONS

The present study was successful in isolating and purifying the CdMV from the infected leaves of cardamom collected from Mudigere, electron microscopic studies of the purified preparations of Katte virus revealed the flexuous rod shaped particles and in standardization of DAC-ELISA and DIBA against CdMV and its detection in tissue cultured plants.

ACKNOWLEDGMENT

We are grateful to the Zonal Agriculture Research Station, Mudigere, Karnataka, India. This work was supported by DBT from the Department of Biotechnology, University of Agricultural Sciences, Bangalore, India.

REFERENCES

[1] Purseglove JW. Tropical Crops: Monocotyledons. Longman group Ltd., UK, 1981; 528-33.

[2] Madhusoodanan KJ, Kuruvilla KM, Priyadarshan PM. Improvement of cardamom. In: Advances in Horticulture**.** Vol. IX, (Eds. K.L. Chadha and P. Rethinam. Malhotra) Publishing House, New Delhi, India, 1994: 307-314.

[3] Venugopal MN. Viral disease of cardamom and their management. *J. Spices and Aromatic Crops*, 1995; 4 (1): 32-39.

[4] Jacob T, Usha R. 3' terminal sequence analysis of the RNA genome of the Indian isolate of cardamom mosaic virus: a new member of genus macluravirus of potyviridae. *Virus Genes*, 2001; 23: 81-88.

[5] Varma PM, Capoor SP. Marble disease of cardamom. *Indian Farming*, 1953; 3: 22-23.

[6] Mayne WW. Report on cardamom cultivation in South India. *Misc. Bull. Imp. Coun. Agric. Res.*, New Delhi, 1951; 50: 58.

[7] Rao DG Katte disease of small cardamom. *J. Plant. Crops*, 1977; 5: 23-27.

[8] Smitha R. Molecular characterization and diagnosis of viruses associated with cardamom mosaic disease. Ph.D. Thesis (Hort.), Univ. Agric. Sci., Bangalore, India.2004: pp. 175.

[9] Gonsalves D, Trujillo E, Hoch HC. Purification and some properties of a virus associated with cardamom mosaic, a new member of the potyvirus group. *Plant Disease*, 1986; 70: 65-69.

[10] Srinivasa R, Narayanaswamy N, Chacko EK, Doreswamy R. Regeneration of plantlets from callus of *Eletteria cardamomum* Maton. *Proc. Indian Acad. Sci.*, 1982; 91 (1): 37-41.

[11] Srinivasulu P, Iizuka N, Rajeshwari R, Reddy DVR, Nayadu MV. Peanut green mosaic virus- A member of the potato virus Y group infecting groundnut (*Arachis hypogea*) in India. *Ann. Appl. Biol*., 1981; 98: 255-60.

[12] Van-Regemortel MHV. Serology and Immunochemistry of Plant Viruses. Academic Press, New York, 1982; pp. 302.

[13] Clark MF and Adams ANCharacteristics of the microplate method of Enzyme Linked Immunosorbant Assay (ELISA) for the detection of plant viruses. *J. Gen. Virol*., 1977; 34: 475-83.

[14] Padmaja AS. Transmission, Purification and detection of field isolates of Cardamom Mosaic Virus (Katte Virus) in Cardamom. M.Sc. (Agri). Thesis. Univ. Agric. Sci., Bangalore, India, 1996: pp. 77.

[15] Tomlinson JA. Purification and properties of lettuce mosaic virus. *Ann. Appl. Biol*., 1964; 53: 95-102.

[16] Ross JP. Purification of soybean mosaic virus for antiserum production. *Phtytopathol*., 1967; 57: 465-67.

[17] Stace-smith R, Tremaine JH. Purification and composition of potato virus Y. *Phytopathol*., 1970; 60: 1785-89.

[18] Damirdagh IS, Shepherd RJ. Purification of the tobacco etch and other viruses of the potato Y group. *Phytopathol*., 1970; 60: 132-42.

[19] Steevenson WR, Hagedorn DJ. Partial purification of pea seed-borne mosaic virus. *Phytopathol*., 1973; 63: 1346-1352.

[20] Gordon DTand Gingery RE Purification of maize dwarf virus by continuous flow centrifugation. *Phytopathol*., 1973;63:1386-1392.

[21] Luisoni E, Boccardo G, Milne RG. Purification and some properties of an Italian isolate of poplar mosaic virus. *Phytopathol*., 1976; 85: 65-73.

[22] Nozu Y, Yamura R. Use of Triton X-100 for isolation of tobacco mosaic virus from some plants other than tobacco. *Virol*., 1971;43: 514-15.

[23] Hull R, Shepherd RJ, Harvey JD. Cauliflower mosaic virus: An improved purification procedure and some properties of the virus particles. *J. Gen. Virol*., 1976; 31: 93-100.

[24] Van-Oosten HJ. Purification of plum pox virus with the use of Triton X-100. *Neth. J. Plant Pathol*., 1972; 78: 33-44.

[25] Randles JW, Harrison BD, and Roberts IM *Nicotiana velutina* mosaic virus: Purification, properties and affinities with other rod-shaped viruses. *Ann. Appl. Biol*., 1976; 84: 193-204.

[26] Nagaraju N. Studies on the influence of certain exogenously applied substances on pepper vein banding virus transmission, multiplication and symptoms in bell pepper (*Capsicum annum L.*), 1996, Ph.D. Thesis, Univ. Agric. Sci., Bangalore, India, 1996: 114.

Source of Financial Support: DBT-GOI

Conflict of interest: Nil

