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SHORT COMMUNICATION

Survey and RT-PCR Based Detection of *Cardamom mosaic virus* Affecting Small Cardamom in India

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Abstract Mosaic or *marble* or *katte* disease caused by *Cardamom mosaic virus* (CdMV) is an important production constraint in all cardamom growing regions of the world. In the present study, 84 cardamom plantations in 44 locations of Karnataka and Kerala were surveyed. The incidence of the disease ranged from 0 to 85%. The incidence was highest in Madikeri (Karnataka) while no incidence was recorded in Peermade (Kerala). In general, incidence and severity of the disease was higher in cardamom plantations of Karnataka. A procedure for total RNA isolation from cardamom and detection of CdMV through reverse transcription-polymerase chain reaction (RT-PCR) using primers targeting the conserved region of coat protein was standardized and subsequently validated by testing more than 50 field cardamom samples originating from Karnataka and Kerala states. The method can be used for indexing the planting material and identifying resistant lines/cultivars before either they are further multiplied in large scale or incorporated in breeding.

Keywords Small cardamom · *Katte* · Mosaic · Incidence · Distribution · *Cardamom mosaic virus* · RT-PCR · Detection

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Small cardamom (*Elettaria cardamomum* Maton) also known as "Queen of Spices" is a perennial herbaceous rhizomatous monocot belonging to the family Zingiberaceae. Cardamom, a native of the moist evergreen forests of Western Ghats of Southern India is valued for its dried capsules [9]. Cardamom industry encounters several production constraints of which katte/mosaic disease incited by *Cardamom mosaic virus* (CdMV) (genus: *Macluravirus*) is the major one in India, Guatemala and Sri Lanka. In monocrop conditions total decline of plants occurs in a span of 3–4 years of infection [5, 12, 14]. The disease is characterized by prominent discontinuous yellowish stripes running out from midrib to the margin of young leaves. Primary spread of the disease is through infected clones while secondary spread occurs through non-persistent transmission by the aphid, *Pentalonia nigronervosa* f. *caladii* [11, 13]. Coat protein and 3′ UTR sequence analyses of different isolates of CdMV revealed the existence of high variability at the N-terminal region [4]. Proper detection of pathogens and use of healthy planting materials are prerequisites for integrated management of diseases. As titre of the virus, seasonal variations, age, resistance and susceptibility of the genotype affects symptom expression, visual diagnosis of viral diseases cannot be employed as a fool proof criterion for identification of the infected plants. Hence, the present study was formulated with the objectives to study distribution and to develop RT-PCR based method for detecting CdMV in plants.

During 2008–2009, 84 cardamom plantations belonging to 44 geographical locations in Karnataka and Kerala were surveyed to study the incidence and distribution of mosaic disease by employing methodology described by Govindaraju et al. [3]. Representative virus isolates (infected plants) collected during the survey were established

Table 1 Distribution and incidence of mosaic disease on small cardamom in Karnataka and Kerala

State/district	Regions/zones	No. of locations surveyed	No. of fields surveyed	Disease incidence	
				Range (%)	Mean (%)
Karnataka					
Hassan	Sakleshpur	8	12	30–73	48.58
Kodagu	Somwarpet	2	3	2–15	10.00
	Virajpet	1	2	17–26	21.50
	Madikeri	2	8	32–85	62.00
Kerala					
Idukki	Udumbanchola	17	37	0–6	0.54
	Peermade	4	4	0	0.00
	Devikulam	5	7	0–12	1.71
Wayanad	Sulthan Bathery	4	8	0	0.00
	Vythiri	1	3	0–2	0.66

under insect proof glass house conditions and subsequently used for developing RT-PCR based detection method for the virus. Total RNA from leaf tissue was extracted as per the protocol described by Bhat and Siju [1] except that TRI reagent (Sigma-Aldrich) was used for initial extraction instead of denaturing solution. Based on multiple alignment of coat protein gene sequences of different isolates of CdMV, conserved regions were identified. Primers (forward: 5′-CACCGATTGCACCAATGAC-3′ and reverse 5′-GAAAACCCACAAAAACTCCC-3′) designed to these conserved regions were used in RT-PCR. The reaction was carried out in 1× *Taq* assay buffer and contained 1.5 mM $MgCl_2$, 10 mM dithiothreitol, 400 µM dNTP mixture, 10 pM each of forward and reverse primers, 1 U of RNase inhibitor, 1.25 U of MMuLV reverse transcriptase, 0.75 U of *Taq* DNA polymerase and 1 µg total RNA as template with a final volume of 50 µl. Prior to the addition of RNA template to the reaction mixture, it was heated to 80°C for 10 min and rapidly cooled down in ice for 3 min to make RNA linear. Single-step RT-PCR was carried out in Eppendorff's Master Cycler Gradient by initially holding the sample at 42°C for 45 min (cDNA synthesis) followed by 94°C for 30 s (denature), 50°C for 1 min (primer annealing) and 72°C for 1 min (DNA synthesis). The whole process except cDNA synthesis was repeated for another 39 cycles and a final extension was allowed at 72°C for 10 min. The reaction products were electrophoresed in 1% agarose gel and visualized under UV light after staining with ethidium bromide. Positive reactions were identified by the presence of 800 bp product specific for CdMV. Absence of the expected amplicon indicated a negative reaction and thus the absence of the virus.

Of the 84 plantations in 44 locations surveyed, incidence of the disease ranged from 0 to 85%, of which highest incidence was observed in Karnataka (Table 1). In Karnataka, of 25 fields belonging to 13 locations surveyed,

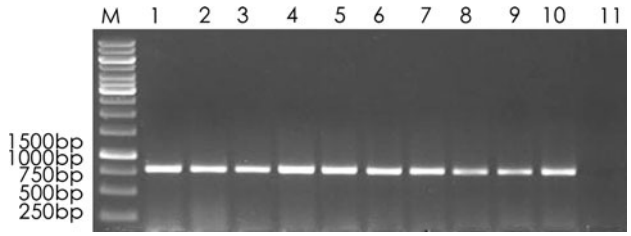

Fig. 1 Detection of CdMV in cardamom samples by RT-PCR. *Lane M*: DNA molecular size markers; *Lane 1–9*: Cardamom samples from different regions; *Lane 10*: Positive control (known infected plant); *Lane 11*: Negative control (known healthy plant)

disease incidence ranged from 2 to 85%. In Kerala, surveys conducted in 59 fields belonging to 31 locations revealed low incidence of the disease. In Idukki, incidence ranged between 0 and 12% while in Wayanad district incidence ranged from 0 to 2%. Results of the survey clearly indicated that the incidence was higher in Karnataka compared to Kerala, which may probably be due to low level adoption of phytosanitary and inadequate plant protection measures [3]. Survey also revealed variation in the symptoms induced by CdMV in different cultivars/varieties cultivated in different geographical locations indicating the occurrence of strains of CdMV in nature which is in agreement with the observations made by earlier workers [4, 6, 8].

RT-PCR was successful in the detection of CdMV in infected plants. The expected size of ~800 bp was observed in infected plants and no such band was observed in healthy plants (Fig. 1). Identity of the amplicon was also confirmed by directly sequencing the gel purified PCR product obtained from four different isolates (Hassan, Kodagu, Idukki and Wayanad) representing Karnataka and Kerala. BLAST analysis of these sequences showed >90% identity with CdMV isolates. RT-PCR method was

Table 2 Detection of CdMV by RT-PCR in field samples collected from different cardamom growing regions of Karnataka and Kerala

State	District	No. of samples tested	No. positive in RT-PCR
Karnataka	Kodagu	29	14
	Hassan	13	12
Kerala	Wayanad	2	1
	Idukki	9	8

validated by testing 53 representative field samples of which 35 tested positive for the virus (Table 2). Early detection of the pathogen is an indispensable component for the effective management of any disease especially those which are incited by viruses. Though detection of CdMV by ELISA was reported by earlier workers [2, 10], RT-PCR based method was not reported so for. Due to sensitivity, accuracy, efficacy and rapidness, PCR-based diagnostics tools are preferred over immunological tools for detecting viruses. In field, the incubation period of CdMV varies from 20 to 114 days during different months of the year [7]. Thus, the RT-PCR based method developed in the present study will be useful in the detection of virus even in infected but asymptomatic plants. The method can be used for indexing the planting material and identifying resistant lines/cultivars before either they are further multiplied in large scale or incorporated in breeding programmes.

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