International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 128-131
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

DETECTION OF CUCUMBER MOSAIC VIRUS IN BRINJAL AND CHILLI BY RT-PCR

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ABSTRACT

Eggplant (Solanum melongena L.) and chilli (Capsicum annuum L.) are the economically important and highly consuming vegetable crops in India and China. These crops were severely affected by many viral diseases in terms of incidence and yield loss. In this study, disease symptoms like mosaic and leaf distortion on brinjal, and leaf puckering and malformation on chilli were observed during the field inspection at cherlopalli village, Chittoor district, Andhra Pradesh (AP). Based on the symptomatology, it was suspected that these plants were infected by Cucumber mosaic virus (CMV). Leaf samples of symptomatic brinjal and chilli plants were collected and tested by RT-PCR with CMV coat protein (CP) gene specific primers and subsequently, the amplified products of ~590 bp in size were commercially sequenced. NCBI- BLAST search results of these sequences confirmed, CMV is the causal agent for the disease in brinjal and chilli plants. The sequence comparison results revealed that the present CMV isolates have 96-99% sequence similarity at both nucleotide and amino acid levels with other isolates of the CMV viz., CMV-KP, CMV TN-Chilli, CMV-B2, CMV-Cucumis, CMV- CLW2, CMV-ZM5 and CMV-SB4 of Capsicum annuum, chilli, banana, Cucumis sativa, cucumber, Nicotiana Tabacum and hot hopper, respectively.

KEY WORDS

Cucumber mosaic virus, Brinjal, Chilli, Reverse transcription (RT)-PCR

INTRODUCCTION

Brinjal/eggplant *(Solanum melongena* L.) and chilli *(Capsicum annuum* L.) are the major commercial solanaceous vegetable crops largely cultivated in China and India for domestic consumption. Both are warm weather crops and mostly cultivated in tropical and subtropical areas. India is the second largest country after China in world brinjal production (12.6 million tonnes in 664 thousand hectares) [1]. In case of chilli, China and India occupied first and fifth places in global chilli production, respectively [2]. Both of these crops were severely infected by several viruses, amongst *Cucumber mosaic virus* (CMV) appears to be the most destructive in terms of incidence and yield loss. CMV belongs to the genus *Cucumovirus*, family *Bromoviridae*, is an economically most important virus infecting 1,200 plant species in over 100 families including di-and monocotyledons around the world [3]. CMV causes great losses in vegetables, cereals, ornamentals and fruits, and it is destructive due to its broad natural host range, non-persistent transmission by many aphid species, mechanical and seed transmission in some hosts [4]. In India, only limited reports were available on molecular characteristics of CMV isolates [5]. CMV is a small isometric (28-30 nm) single-stranded positive-sense tripartite ssRNA virus [6]. Most of the isolates of CMV consist of three genomic RNA species namely RNA1, 2 and 3 and one subgenomic RNA. CMV has been divided into IA, IB and II subgroups and Asian strains including Indian have been grouped under subgroup IB [7]. Based on recent reports, CMV subgroup I was found to be predominant in tropical and subtropical regions, whereas subgroup II was more frequently found in

temperate regions [8]. Mild mosaic, mottling, shoe string, fern leaf, vein bending, vein clearing and leaf deformation were characteristically observed as prominent symptoms in the naturally infected chilli plants [9] similarly, the CMV infected brinjal plants show mild mosaic, mottling of leaves and reduced growth. CMV can be detected by observing the local or systemic symptoms on test/diagnostic hosts through mechanical inoculation. CMV causes chlorotic or necrotic lesions on local lesion hosts like *Chenopodium amaranticolor, C.quinoa*, *V. unguiculata* and cause systemic symptoms like mosaic, mosaic with fern leaf symptoms on *C.sativus* and *Lycopersicon esculentum*. Although indicator plant assays have been largely supplemented by other diagnostic techniques, they may still be useful in situations where other methods are unavailable. Serological diagnostics including ELISA, Dot-ELISA, Western blotting were used to detect CMV in sevaral host crops. Polyclonal (PAbs) and monoclonal (MAbs) were produced to differentiate CMV strains into at least three serogroups. Similarly, nucleic acid-based diagnostics like RT-PCR, real-time PCR, IC RT-PCR, RT-PCR-RFLP, Microarray and Luminerxx TAG were developed to detect and discriminate CMV isolates in various host plants [10-15]. In the present study, we have selected RT-PCR as a diagnostic tool to diagnose CMV infection in brinjal and chilli crops.

MATERIALS AND METHODS

Total RNA isolation

The symptomatic brinjal and chilli plants suspected for CMV infection were collected from fields of cherlopalli village of Chittoor district,AP and used for RNA isolation. The leaves of the plants were washed initially with distilled water and later with RNase free sterile distilled water and macerated in a RNase free mortar and pestle by adding liquid nitrogen. Approximately 100 mg of leaf material was used for isolation of total RNA by following the Qiagen total RNA isolation kit method. Finally, the column was placed in a new 1.5 collection tube and RNA was eluted in 30 µl of RNeasy-free water.

RT-PCR and sequencing

Initially reverse transcription step was performed to synthesize cDNA of viral RNA. To a 20 µl reaction, 1 µg of total RNA and 0.5 µg of CMV-CP specific reverse primer (10pM) were added into a RNase-free 0.2 ml eppendorf tube and incubated at 70°C for 5 min and chilled on ice. After incubation, reagents i.e., 5x reaction buffer, 10 mM dNTPs mix and 200 U of M-MuLV reverse transcriptase were added as described in the manual (Thermo Scientific) and incubated for 1 h at 42°C. The reaction was stopped by heating the mixture for 10 min at 70°C. The resulted cDNA was used for PCR amplification by using the CMV-CP gene specific primers. A 50 µl reaction was set in a 200 µl eppendorf tube, the ingredients, 5x reaction buffer,5.0 µl; 25 mM MgCl2, 4.0 µl; dDNTs mix,1.0µl; primers (Forward: 5'ATGGACAATCTGAATCAC3' and Reverse: 5'TCAAACTGGAGCACCC3') (10pM) each, 2.0 µl; Taq.polymerase,0.5 µl; cDNA template,5.0 µl and double distilled water,30.5 µl were added. The cyclic conditions were, denaturation, 94°C/20s; annealing 50°C/30s and extension,72°C/1min for 35 cycles. After completion of the reaction the amplicons were run on 1% agarose gel and analyzed under gel documentation system. The amplified CP gene of CMV was cloned into a cloning vector i.e., pTZ57R/T (Thermo Scientific) and transformed into *E.coli* bacterial strain (DH5α) and positive colonies were confirmed by *Eco*RI and *Bam*HI restriction enzymes. The confirmed positive clones were sequenced by commercial sequencing facility (MWG, Bangalore) and the resulted sequences were subjected to NCBI-BLAST search tool to confirm the pathogen. Further, the CP sequences of the present CMV isolates were compared with other Genbank reported CMV isolates by ClustalW multiple sequencing tool to know the sequence similarity at nucleotide and amino acid levels.

RESULTS AND DISCUSSION

CMV is a widely prevalent plant virus causes major economic losses in many agricultural and horticultural crops around the world. It has the largest host range of all plant viruses, spreads quickly by means of many aphid vectors and mechanical transmission. It can infect both mono and dicot plants including vegetable, fruit, cereal and ornamental crops. In the present study, we have noticed some of the plants in the growing brinjal and chilli crops at cherlopalli village were severely infected and expressed characteristic symptoms like mosaic and leaf distortion on brinjal, and leaf puckering and malformation on chilli. Based on these symptoms initially it was suspected that CMV probably the causal agent for the disease. Field survey in *kharif* and *rabi* seasons, the systemic symptoms on naturally infected plants were mosaic and leaf distortion on brinjal,

chlorotic spotting, mosaic, leaf puckering and malformation on chilli [16]. However, symptom-based disease diagnosis is always not preferable, it misleads from actual pathogen for the disease. To detect accurate virus for the disease, various formats of sero and molecular diagnostics have developed. ELISA is one of the reliable serodiagnostics; however, it can have limited detection sensitivity with very low virus concentration [17]. RT-PCR is one of the highly sensitive and efficient diagnostic tools than ELISA in detection of viruses [18]. Hence, in the present study RT-PCR was selected as a diagnostic tool to diagnose the symptomatic plants for CMV. The symptomatic leaf samples of chilli and brinjal (5 samples from each crop) were randomly collected from the fields and subjected to RT-PCR by CMV-CP gene specific primers. A sharp DNA band of ~650 bp in size was amplified in both chilli (3 samples) and brinjal (5 samples) samples. Approximately 650 bp in size DNA band was amplified in CMV infected chilli plants [19]. Similar size of DNA band was obtained from egg plants infected with CMV [20]. Further, amplicons of CMV-CP gene were cloned into pTZ57R/T vector and transformed into *E.coli* bacterial strain (DH5α) and positive colonies were confirmed by *EcoR*I and *BamH*I restriction enzymes. The selected clones (2 clones of chilli and brinjal) were subjected for commercially sequencing and these resulted sequences were analyzed by NCBI-BLAST search tool. It revealed that the both sequences were CMV, and these sequences were submitted to NCBI Genbank as CMV-BR1 (HM348785) and CMV-Chil (HM348786) isolates. Multiple sequence comparison of these two sequences with other CMV isolates viz. CMV-KP (KM272276) of *Capsicum annuum*, - TN Chilli (KJ645896) of chilli, -B2(HM348784) of banana, -BK16(FN552546) *Cucumis sativa*, -CLW2(JN054635) of cucumber, -ZM5(KJ746023) of *Nicotiana Tabacum* and -SB4(k7746021) of hot hopper, revealed about 96 to 99% sequence similarity at nucleotide and amino acid leaves. The derived CP sequences shared 96 and 95% nucleotide and 98.6 and 99.5% amino acid sequence identities with corresponding sequences of CMV isolates from banana and eggplants, respectively, from India [21]. Sequence similarity between the present two isolates ie., CMV-BR1 and CMV-Chil have identified 98% at nucleotide and amino acids level. It was found that many weed like *Euphorbia hirta* and *Tridox procumbens* were naturally growing nearby brinjal and chilli crops at samples collected area. These weeds can act as alternative natural host for CMV and play very important role to carry the disease into the subsequent crops. CMV was also detected from naturally infected weed hosts around tarai region in Uttar Pradesh [22]. Elimination of weed hosts and control of aphid vectors can control CMV incidence in the crops. To conclude, the causal agent for the disease in naturally infected chilli and brijal plants was confirmed as CMV by RT-PCR. Hence, RT-PCR is one of the highly sensitive, reliable and efficient diagnostic techniques to detect CMV in solanaceous plants.

Table 1. Nucleotide sequence comparison among CMV isolates of vegetable crops.

Isolate Sequence	CMV -Chil	CMV-KP	CMV-TN Chilli	CMV-BR1	CMV -B2	CMV-BK16	CMV -ZM5	CMV-SB4	CMV-CLW2
CMV-Chil	ID	98.9	98.9	97.8	97.8	97.4	97.2	97.2	97.1
CMV-KP		ID	98.7	97.8	97.8	97.5	97.4	97.1	97.2
CMV-TN Chilli			ID	97.8	97.8	96.9	96.8	96.4	96.6
CMV-BR1				ID	99.0	963	96.4	96.1	96.3
CMV-B2					ID	96.0	96.1	96.1	96.0
CMV-BK16						ID	97.4	97.4	98.1
CMV-ZM5							ID	98.4	98.0
CMV-SB4								ID	97.7
CMV-CLW2									ID

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Received:03.05.18, Accepted: 04.06.18, Published:01.07.2018

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