"VIRUS INDEXING USING MOLECULAR MARKERS"

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Abstract

As part of the study, infected leaf samples were collected from the cassava fields, Thiruvananthapuram and Alappuzha. Symptom scoring was done according to Hahn et al 1984, and it was found that CMD is widespread in the field having very high infection rates and incidence. Mixed infection (both ICMV and SLCMV) obtained from one sample explains that the situation is crucial as there might be a chance of recombination between these two viruses to evolve into more virulent strains and cause devastating yield loss. So, efficient management is inevitable to control this devastating disease from fields. Methods like Phyto sanitation (selection of disease-free planting material),proper disposal methods The electrophoretic banding profile of cassava proteins provided a powerful tool for estimating diversity that exists in the genotypes.

Key Words: SDS-PAGE , ICMV , SLCMV, CMD.

Introduction

Cassava (*Manihot esculenta Crantz*), popularly known as tapioca, is cultivated in 102 countries. It is an important staple food for more than 500 million people in the tropics, apart from being an industrial crop. Cassava mosaic disease (CMD) is caused by viruses belonging to the genus Begomoviral of the family Geminiviridae, which are characterized by small, geminate particles containing circular, single-stranded DNA molecules (Briddon and Markham, 1995). Twenty different viruses have been reported from cassava (Thresh et al., 1994). Among these, in India, cassava mosaic disease is caused by two viruses – Indian Cassava Mosaic Virus (ICMV) and Sri Lankan Cassava Mosaic Virus (SLCMV) (Malathi et al., 1985; Dutt et al., 2005).Cassava is a major staple food in the developing world, providing a basic diet for over half a billion people. It is one of the most drought-tolerant crops, capable of growing on marginal soils.

Fig 1: CMD infected cassava plant in the field Whitefly *Bemisia tabaci*.

Sample Collection
Samples of cassava (*Manihot esculenta* crantz) (both infected and healthy leaves) were collected fromtwo locations in Kerala namely Thiruvananthapuram and Alappuzha. These samples were photographed, (Figure no.1), placed individually in the pre-sterilized polythene bags, sealed and kept in a portable ice chest and transported to the laboratory for further molecular analysis.

Total genomic DNA isolation from samples
Virus indexing is a technique used in virology to confirm the presence of particular viruses in plants using polymerase chain reaction using specific primers for particular viruses. In this experiment virus indexing was done to confirm the presence of cassava mosaic virus in field infected samples using multiplex PCR for the differential identification of ICMV and SLCMV.For this purpose, three primers (ICMV specific forward primer, SLCMV specific forward primer and a common reverse primer) should be used for the detection of these two species of cassava mosaic viruses (ICMV and SLCMV) using a single PCR (multiplex PCR) with total genomic DNA isolated from these samples. Therefore, DNA isolation is a prerequisite wherein the isolated DNA can be PCR amplified using these primers and the products can be analysed for the differential detection of ICMV and SLCMV. For the same, good quality total genomic DNA was isolated from the collected leaf samples using CTAB method.
CTAB method of DNA isolation

Analysis of the extracted DNA
Agarose gel electrophoresis
The integrity and quality of the extracted DNA was checked in agarose gel electrophoresis after CTAB DNA isolation. An agarose gel of 0.8 % was prepared in 1X TAE buffer and ethidium bromide (EtBr) was added with a final concentration of 0.5 µg/ml. An aliquot of the DNA sample (2 µl) mixed with 3X loading dye was loaded in each of the wells of the gel. The gel was run at 80 V for 10 min. The gel was then visualized under UV transilluminator for checking the quality of DNA (Figure no.6). Concentration of isolated DNA were determined using Nano spectrophotometer and are formulated in Table no. 1.

Differential identification of ICMV and SLCMV using multiplex polymerase chain reaction
Polymerase chain reaction: In order to analyse the species of cassava mosaic virus present in the samples, multiplex PCR with three primers is used in the present study. From the total genomic DNA isolated from leaves of each sample, DNA at a concentration of 25 ng/µl were used for Polymerase Chain Reaction (PCR) using three primers. The sequences of primers used in PCR analysis were tabulated in Table no.PCR was carried out in Eppendorf Nexus Master cycler. PCR programme was set with initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for45 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min. Final extension was done at 72°C for 5 min. Control reactions were carried out to distinguish the target products from non-target products and primer dimer. The amplified products (600bp for SLCMV and 900bp for ICMV) along with PCR Marker (1 kb plus) from 'Thermo Scientific' were loaded on agarose gel (1%). The gel was run at 80 V for 45 min. The gel was then visualized under UV transilluminator for analysing the results.

Results
In the present study three samples of cassava (*Manihot esculenta* crantz) were collected from two locations in Kerala namely Thiruvananthapuram and Alappuzha, to identify the type of cassava mosaic virus (differential identification of ICMV and SLCMV) present in these samples using multiplex PCR.

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Sample Collection

Figure no. 2: Cassavasamples collected for the study. a- cassava leaves infected with cassava mosaic disease, b- healthy leaves of cassava

Table no2: Details of multiplex primers used for the study

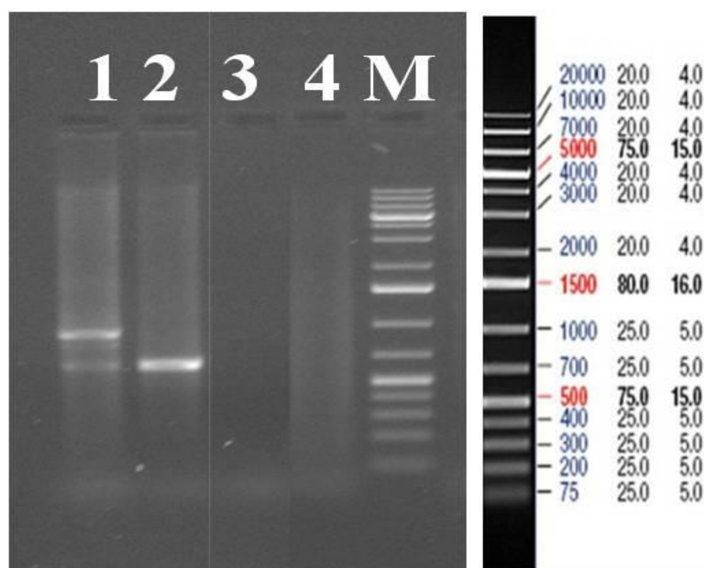

Figure no. 7: PCR product of genomic DNA, isolated from leaf samples of cassava samples using multiplex primer set.1- Alappuzha infected sample, 2- Alappuzha healthy sample, 3- Thiruvananthapuram infected sample, 4- Thiruvananthapuram healthy sample, M- 1kb plus DNA marker.

Sample no.	Band size (in base pair)
1	600 and 900
2	600
3	No
4	No

Table no 3: Multiplex PCR results of samples used for the present study. 1**-** Alappuzha infected sample, 2- Alappuzha healthy sample, 3- Thiruvananthapuram infected sample, 4- Thiruvananthapuram healthy sample

Conclusion

Virus indexing is a technique used in virology to confirm the presence of particular viruses in plants using polymerase chain reaction using specific primers for particular viruses. In this experiment virus indexing was done to confirm the presence of cassava mosaic virus in field infected samples. Samples of cassava (*Manihot esculenta* crantz) (both infected and healthy leaves) were collected from two locations in Kerala namely Thiruvananthapuram and Alappuzha. The collected samples were placed individually in the pre-sterilized polythene bags, sealed and kept in a portable ice chest and transported to the laboratory for further analysis.

Sample no.	Virus infection
1	Both ICMV and SLCMV infection
2	SLCMV infection
3	No viral infection
4	No viral infection