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Research Article

Detection of Jatropha Mosaic Indian Virus (JMIV) on Jatropha Plant (*Jatropha Curcas*)

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A B S T R A C T

Jatropha plant is a major crop for biofuel production in countries such as India. The recent increase in the cultivation of Jatropha has resulted in the manifestation of pest and disease attacks on the crop, including jatropha mosaic Indian virus (JMIV) which is a member of the genus Begomovirus (family Geminiviridae). Jatropha leaves were collected from Bangalore, South India showing mosaic symptoms, malformation of leaves and chloric specks. Total DNA was extracted from infected leaves and the extracted DNA was subjected to PCR to amplify JMIV DNA using degenerate primers and subsequently with begomovirus-specific primers for both the DNA-A (JMIV-A 970F and JMIV-A 970R) and DNA-B (JMIV-B 970F and JMIV-B 970R). The PCR products obtained with degerate primers were ~ 530bp which further confirmed the association of a begomovirus with jatropha. Furthermore, PCR products in the amplification of both full-length DNA-A and DNA-B components of JMIV were successful for the first time which produces ~ 2.8 kb on both the genome.

Keywords: Core Coat Protein, Polymerase Chain Reaction, JMIV, Ultra Violet Light

Introduction

Jatropha (*Jatropha curcas*) is one of the 170 known species that belong to the family *Euphorbiaceae*.[1] The plant is also called as physic nut which has a pale-green leaves and it produce latex when the plant is cut.[1] The jatropha plant derived it name from the Greek word iatros (doctor) and trophe (food) which indicates the plant's medicinal value.[1] The jatropha plant usually grows to a height of 3-4 meters, but under favorable agronomic condition it can grow up to 8-10 meters.[2] The plant is also a drought resistance; however, the cultivation of jatropha has increased dramatically because of its economic importance of seed as raw material for biofuel synthesis.[2] The plant is also useful as a source of organic manure, a medicinal plant, and a source of lubricant and also used in soap making. Only recently with the development of biofuel industry has jatropha used as a source of income in Nigera.[3] Today jatropha is cultivated intensively in many developed and developing countries which have led to an increased number of pest and diseases affecting the plant including begomoviruses which cause jatropha mosaic disease (JMD). JMD was first reported from Puerto Rico on Jatropha *gossipiifolia* and it was also present in Jamaica, Mexico, Mali, Cuba and

America.[4] Furthermore, JMD was also reported in 2004 from India, and is associated with Jatropha mosaic Indian virus (JMIV) which is transmitted, by *Bamisia tabaci*.[5] Based on sequencing of the partial genome of JMIV, it is closely related cassava mosaic viruses infecting cassava in India and Nigeria.[5]

A begomovirus is the causative agent of jatropha mosaic disease and thus as a member of the genus Begomovirus belong to the family Geminiviridae.[6] Begomoviruses DNA are super coiled in nature with a size of about 20-35nm.[7] Begomovirus have single-stranded DNA (ssDNA) genomes, which are circular in form. While some begomoviruses have bipartite genome, which are referred as DNA-A and DNA-B components.[6,8,9] Some others are monopartite in nature as they have only one circular single-stranded DNA-A. Some monopartite viruses have an additional satellite molecule known as DNA-β.[10]

Begomoviruses are transmitted by insect vectors called whiteflies of the species complex *Bemisia tabaci* (B. *tabaci*),[3,11] which belong to the family *Aleyrodidae*, order *Hemiptera*. Whiteflies are usually found in tropical and subtropical region[12] and they cause significant economic damage in the agricultural sector world-wide.[10,13]

Materials and Methods

Detection of Begomoviruses

Plant sample of jatropha leave showing jatropha mosaic disease symptoms were obtained from Bangalore, South Indian in March 2008. The fresh samples were stored in NRI's - 80^{0}C freezer while dry samples were stored at room temperature. Total DNA was extracted from infected jatropha leaves, using[14] adaptation of the CTAB method of Lodhi.[15] PCR were set up for detection of begomoviruses, using Deng A and Deng B primers (Deng-A primer TAATATTACCKGWKGVCCSC, Deng-B primer TGGACYTTRCAWGGBCCTTCACA.[16] These primers amplified ~ 530bp PCR product within the intergenic region (IR) of the coat protein gene of begomoviruses.

PCR were carried out in a 25 µL reaction, containing SDW, 10x PCR buffer, 25mm $MgCl_2$, 2.5mM dNTPs, 20µM primers, 5U/ µl *Taq* polymerase and DNA sample for the detection of begomoviruses. The reactions were carried out in a thermo cycler marching, the temperature cycles for PCR at the initial 10 cycles are denaturation temperature was 94^{0}C for 2 min, annealing temperature was 55^{0}C for 45 sec, at the extension stage the temperature was 72^{0}C for 1 min. The subsequent cycles were all most the same temperature; the cycle was ended at 20^{0}C hold. The PCR product and 1kb Gibco-BRL molecular marker were than separated by electrophoresis on 1% agarose gels, which were stained with 1% ethidium bromide solution. The bands were visualized under UV light.

Amplification of Full-length DNA-A and DNA-B for Detection of JMIV

JMIV specific primers for amplifying full-length DNA-A were design using the software package BioEdit downloaded using the core CP sequence of JMIV DNA-A. Since DNA-B sequence were not available for JMIV, sequence of its nearest relative's *Indian cassava mosaic virus* and *Sri Lankan cassava mosaic virus* were obtained from the database and aligned using BioEdit. The primers for JMIV-A (970R) are ACCGGTGGTCAGTATGCT and JMIV-A (970R) ACCGGTGACAGTCGCA, for JMIV-B (970F) ACTAGTTTTAAAGATGATGG, JMIV-B (970R) ACTAGTCCAGATAGGGTATC.

PCR set up for DNA-A and DNA-B are same only the virus specific primers were differing. The PCR master mix reaction contains the following ingredients in 25 µL Eppendorf tube, the reaction containing SDW, 10x PCR buffer, 25mm $MgCl_2$, 2.5mM dNTPs, 20µM primers, 5U/ µl Red hot polymerase and DNA sample. The temperature cycles for PCR, are at the initial denaturation of 94^{0}C for 1min, while the following 35 cycles, were 94^{0}C 1min, 50^{0}C 1min. 72^{0}C 3min, ending with 72^{0}C 10min and hold at 15^{0}C.

Results

Amplification of full-length DNA-A and DNA-B of JMIV

The full-length DNA-A and DNA-B genome components of JMIV were amplified using JMIV-A 970F and JMIV-A 970R primers for DNA-A and JMIV-B 970F and JMIV-B 970R for DNA-B primers. However, both the primers amplified PCR product of expected size of ~ 2.8Kb, the PCR products were analyzed in 1.2 % agarose gels as shown below:

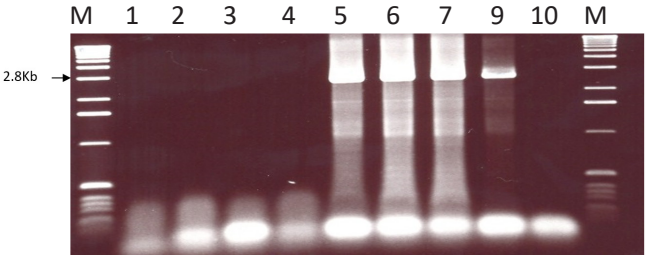

Figure 1.Gel electrophoresis of PCR amplified full-length DNA-A component with the used of JMIV-A 970F and JMIV-A 970R primers. Lane 1- 4 are undiluted DNA samples while lane 5 – 9 are the same sample amplified four time in order to have enough PCR product for further research, the lane 10 is the nagetive control. M is the molecular weight marker (1Kb)

The above DNA samples on Figure 1, were futher amplified with the used of DNA- B specific primers at 52^{0}C annealing temprature which also a successefull PCR, the PCR product were sperated in 1.2 % agarose gel as shown below:

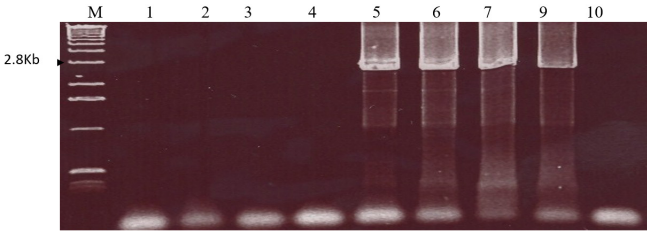

Figure 2.Gel electrophoresis of PCR amplified full-length DNA-B component with the used of JMIV-B 970F and JMIV-B 970R primers. Lane 1-4 are undiluted DNA samples while lane 5-9 are the same sample amplified four time in order to have enough PCR product for further research, the lane 10 is the nagetive control. M is the molecular weight marker (1Kb)

Discussion

Jatropha plant samples were collected from Bangalore in India. The plant samples have a mild mosaic, chlorotic specks on the leaves and malformation of the leaves with severe reduction in the size of the leaves which are all symptoms of the virus (JMIV), that cause by begomoviruses.[11]

Total DNA was extracted from the jatropha leaves samples, using modified CTAB method. The extracted DNA samples were amplified with the used of degenerate primers (Deng-A and Deng-B primers) that can amplify ~ 530 bp within the coat protein (CP) of begomovirus.[16] PCR products of about 530bp were obtained which indicates that JMIV is caused by begomovirus. Moreover, the result obtained agreed with the results of Narayana HA and Seal SE et al.[5,11] were they obtained a PCR product of 570bp from jatropha infected samples and also it supported by the work of Raj SK,[17] which they confirm the association of begomovirus with JMV by obtaining 550bp of PCR product with the used of Deng primers.

The primary aims of this work is to validate the molecular diagnostic method for JMIV and further characterization of the virus by virus genome sequencing with relation to other virus sequence in Gen-bank. In order to achieve these goals, primers were design that can amplify full-length DNA-A and DNA-B of JMIV. The primers are JMIV-A 970F and JMIV-A 970R for DNA-A and JMIV-B 970F and JMIV-B 970R for DNA-B. PCR product of DNA-A fragment was amplified with the used of design primers that produces 2.8 Kb of the PCR products as indicated in Figure 1. The amplification of DNA-B fragment with design primers produced an intense band on sample five only (Figure 2) which also produces 2.8 kb of the PCR product.

Conclusion

The PCR analysis of the virus (JMIV) with degenerate primers and virus specific primers confirm the association of jatropha plant with bipartite genome of begomovirus.

The aim to identify the Jatropha mosaic Indian virus of DNA-A and DNA-B genome components of the virus was successful that produces ~ 2.8 Kb of the PCR product in both the genome of the virus.

Further analysis needs to be undertaking in order to know the complete nature of the virus and its epidemiology, for effective control of the virus, considering the global economic important of the crop (jatropha plant) for biofuel production.

Conflict of Interest: None

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