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Molecular detection of *Begomovirus* (family: *Geminiviridae*) infecting *Glycine max* (L.) Merr. and associated weed *Vigna trilobata*

S. V. RAMESH, B. S. CHOUHAN AND R. RAMTEKE

ICAR-Indian Institute of Soybean Research (ICAR-IISR),
Khandwa Road,Indore 452 001, Madhya Pradesh

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ABSTRACT

Plant virus detection and diagnosis play major role in ascertaining the crop losses and to devise efficient disease control strategies. Yellow mosaic viruses (genus: Begomovirus; family: Geminiviridae) are serious pathogens of grain legumes and leguminous weeds. In this study, we report PCR based diagnosis of legume yellow mosaic viruses infecting soybean and associated weed species Vigna trilobata in soybean crop ecosystem. Simple and efficient PCR based detection designed for studying begomoviruses infecting soybean was utilized in identifying yellow mosaic virus infection in weed Vigna trilobata. Initial PCR detection demonstrated yellow mosaic symptoms in Vigna and soybean are due to legume begomovirus infection. Further PCR assay in Vigna and soybean revealed infection was due to Mungbean yellow mosaic India virus (MYMIV) which is prevalent in soybean grown regions of central India. Implications of MYMIV infection in perennial weed species like Vigna for disease management strategies are also discussed.

Keywords : Begomovirus, detection, PCR, weed host

Grain legumes such as soybean, urdbean, mungbean, and field bean are major source of dietary protein in Indian sub-continent. However, grain legumes suffer extensive losses due to widespread virus infections. Among the major viral infections in legumes, losses due to begomoviruses that are collectively called as legume yellow mosaic virus (LYMVs) are economically significant (Varma and Malathi, 2003). Furthermore, detection and diagnosis of LYMVs is an important area of research because virus species causing damage to the cultivation of legumes are diverse eventhough apparent symptomatic differences are not observed in the field conditions. Soybean is an important legume crop grown mainly in the central Indian region and it supplies over 25 Per cent of vegetable oil requirement of the country and a substantial quantum of protein-rich bye product *ie*) de-oiled cake for animal feed requirements (Soybean News, 2016).

Legume yellow mosaic viruses (LYMV) belong to genus *Begomovirus* of family *Geminiviridae*. LYMVs are transmitted by insect vectors) whiteflies and cause severe yellow mosaic disease (YMD) in tropical and sub-tropical conditions. The YMD of legumes in SE Asia is caused due to four species of Begomoviruses such as, *Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic India virus* (MYMIV), *Dolichos yellow mosaic virus* (DoYMV) and *Horsegram yellow mosaic virus* (HgYMV) and are collectively called as legume yellow mosaic viruses (LYMVs) (Fauquet and Stanley 2003; Qazi *et al.,* 2007). The disease is a major constraint in realising productivity potential of legumes in the field conditions. The economic crop loss caused by the infection of yellow mosaic virus accounts for 300 million US $ in all the legumes, including soybean, mungbean, urdbean, field bean *etc*., (Varma and Malathi, 2003).

Begomoviruses are characteristically twinned icosahedral particles which encase the single-stranded DNA genomic components of the virus. The genomes of legume infecting begomovirus in general are bipartite in nature) comprise two ssDNA genomic components namely DNA-A and DNA-B each approximately of 2700 nucleotides long.

Detection and diagnostics of yellow mosaic virus infection in legumes is imperative to develop appropriate disease control strategies. In addition, species of begomovirus causing the disease in soybean and other legumes are diverse although symptomatic differences are rare. In Northern and Western parts of India *Mungbean yellow mosaic India virus* and *Mungbean yellow mosaic virus* cause the disease respectively (Usharani *et al*., 2004; Girish and Usha 2005; Ramesh *et al.,* 2013). Further, incidence of LYMVs such as Horse gram yellow mosaic virus and Dolichos yellow mosaic virus is also reported (Maheshwari *et al.,* 2014). Incidence of LYMVs in wild species of legumes was also documented (Naimuddin and Pratap, 2011). The present study aims to investigate and detect begomovirus species infecting soybean and associated weed species *Vigna trifoliata* that are found growing in the ICAR-Indian Institute of Soybean Research (ICAR-IISR) research farm.

MATERIALS AND METHODS

Leaves of soybean and *Vigna trifoliata* that showed typical yellow mosaic symptoms were collected from

Email: ramesh.sv@icar.gov.in

the research farm ICAR-IISR, Indore. Infected plants were identified based on characteristic disease symptoms and active presence of whiteflies-insect vectors of begomoviruses. Rapid DNA extraction protocol suitable for small amount of plant tissues was followed to isolate total DNA from infected and healthy leaf samples (as negative control) (Doyle and Doyle, 1987).

Total DNA extracted was used as a template in polymerase chain reaction (PCR) to detect the occurrence of legume infecting begomovirus. Oligonucleotides used for detecting legume begomoviruses are RUGEMF1 5' TGTGAGGGACCATGTAAAGTTC 3' and RUGEMR1 5' GCATGAGTACATGCCATATAC 3'. Similarly, PCR amplification of partial DNA-B region was carried out with following primers R-5: 5'ATGTTTACTCGTAATTATCGCA 3' and R6 5' TTATCCAACGTATTTCAATTCA 3' using template DNA isolated from soybean. PCR studies were carried out to determine the species of legume begomovirus infecting soybean and *Vigna trilobata* using primers MYMV F 5' GTGTTAAGTCTATCTGGG 3', YMV R 5'CACAGGATTTGATGCATGAG 3' and MYMIV F 5' GCATCAAGTCCGTGTACATTAC 3' (Ramesh *et al.,* 2016).

The PCR reaction mixture comprised 50ng of DNA template, forward and reverse primers (10µM), 1µl of dNTPS (10 mM), 1µl of $MgCl_2$ (25mM), 1 U of *Taq* polymerase (New England Biolabs). The thermal cycler conditions for virus gene amplification involve 1 cycle of DNA denaturation at 94°C for 5 min followed by 30 cycles each having a denaturation at 94°C for 30 sec. annealing (50-57 °C for 40 seconds depending on the PCR amplicons) and a primer extension at 72°C for 30 sec followed by final extension of 72°C for 5 min.

Restriction enzyme digestion analysis of partial DNA-B amplicons was carried out in a 20 µl reaction in a reaction mixture comprising of 500 ng of PCR amplicon with 5 units of restriction endonucleases like *Hin*d III, *Pst* I, *Nco* I. The products restriction enzyme digestion analysis was resolved in 2 Per cent agarose gel electrophoresis in 1X TAE buffer and an aliquot of 100ng of 1 Kb DNA ladder (Fermentas, Massachusetts, USA) used as molecular size marker and visualized by ethidium bromide staining.

RESULTS AND DISCUSSION

In this study, we devised a detection and diagnosis of begomovirus species infecting soybean and associated weed *Vigna trilobata* found in the soybean ecosystem (Fig. 1).

In order to establish the Begomovirus infection, DNA isolated was used as template in amplifying the conserved coat protein gene (AV1) of legume infecting begomovirus. Thermocycler conditions were standardized to obtain amplification of virus genome specific regions from the background pool of plant genomic DNA using primers RUGEMF1 and RUGEMR1. A gradient PCR with annealing temperature range of (Tm 48 to 59°C) was set up to obtain the desired amplification. The PCR conditions at an annealing temperature of 55°C yielded an expected amplicon size of ~ 500 bp in both soybean and *Vigna trilobata* (Fig.2).

PCR based detection revealed presence of legume begomovirus infection and DNA from those positive leaf samples were further subjected to PCR reactions to diagnose the species of begomovirus infecting soybean and *Vigna* samples. We have already reported PCR based assay to differentiate major begomoviruses Mungbean yellow mosaic India virus and Mungbean yellow mosaic virus infecting soybean in India (Ramesh *et al.,* 2016). Following the same protocol we detected for the presence of both the species of virus in soybean and *Vigna trilobata*. Results indicate that none of the samples of soybean and *Vigna* showed amplification specific to MYMV however DNA samples were positive for the presence of MYMIV (Fig.3). It demonstrated that soybean and *Vigna trilobata* were infected with MYMIV the species of begomovirus that was found to be prevalent in Central India region (Ramesh *et al.,* 2016).

In soybean, further confirmatory study was conducted by performing restriction digestion of PCR amplicon from DNA B. Restriction enzyme treatment of DNA B derived PCR product with *Nco*I, *Hin*d III, *Pst* I identified the Begomovirus species inflicting damage on soybean (Fig. 4). *Nco* I digestion resulted in two equal fragments of DNA-B partial region suggesting that the infection is owing to legume infecting begomovirus as *Nco* I recognition site has been found to be conserved in all legume infecting begomoviruses. Restriction of partial DNA-B region with *Pst*-I alone and not with *Hin*d III suggested that the virus is MYMIV as *Pst*-I recognition site had been proven to be conserved in MYMIV (Usharani *et al*., 2004).

Presence and detection of MYMIV in weed species *Vigna trilobata* in soybean ecosystem is concern for soybean growers. Vigna species are hardy and perennial hence withstand adverse weather conditions. It is highly likely that *Vigna* sp. would serve as an alternate host of virus inoculum for YMD in soybean. However, no reports or experimental evidence exist to support this view point. In this context, it is pertinent to control weeds like *Vigna* sp. in soybean fields to eradicate virus inoculum.

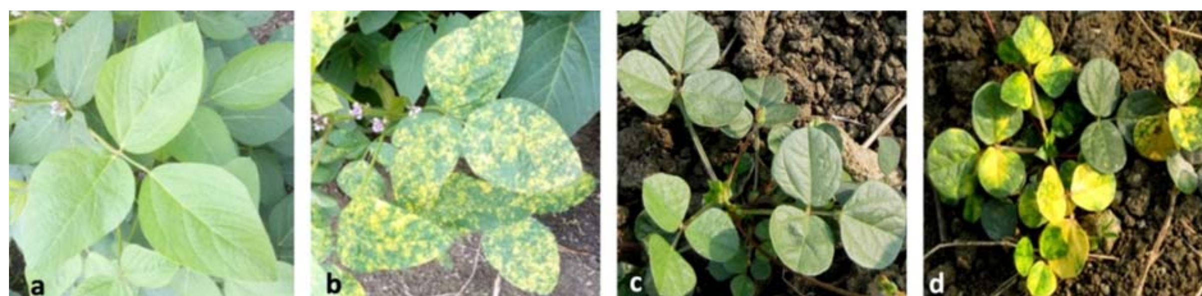

Fig. 1: Healthy and Yellow mosaic disease (YMD) affected plant samples of soybean (a & b) and *Vigna trilobata* (c & d) observed in the fields of ICAR-IISR research farm.

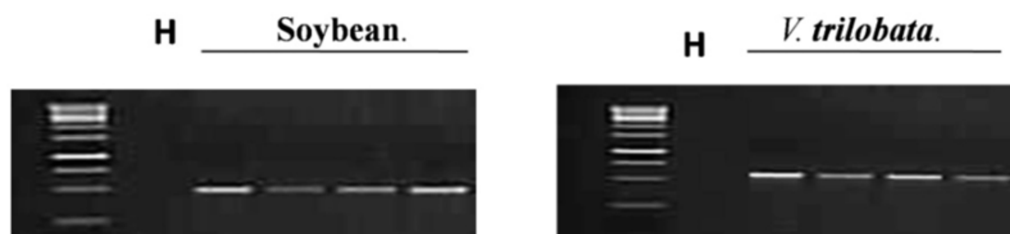

Fig. 2: PCR detection of legume begomovirus in DNA samples extracted from a) soybean and *Vigna trilobata* using RUGEMF1 and RUGEMR1 (Usharani et al., 2004) primers. Amplicons of 505bp were obtained in both soybean and Vigna plants species where H represent healthy leaf samples.

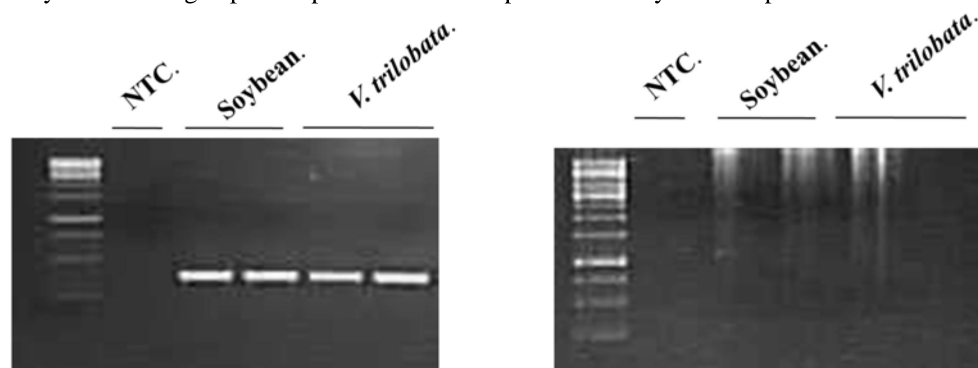

Fig. 3: PCR assay to detect species of begomovirus infecting soybean and *Vigna trilobata* using MYMIV F, and YMV R and MYMV F and YMV R primers in two different reactions were carried out. MYMIV specific amplification was observed in both plant species. NTC represent non-template control where sterile distilled water was used in place of DNA template.

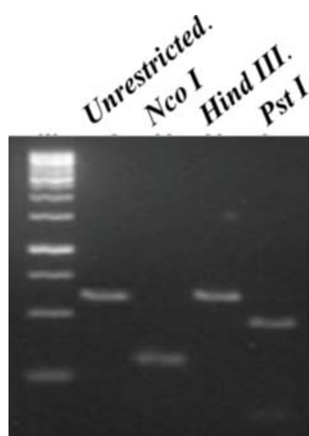

Fig. 4: Restriction enzyme digestion pattern of partial DNA-B genomic region from infected soybean plant. MYMIV specific digestion pattern was observed as the DNA B region was found to be digested only with *Pst* I and *Hin*d III digestion did not reveal any change.

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