IDENTIFICATION AND TRANSMISSION OF HORSEGRAM YELLOW MOSAIC VIRUS CAUSING YELLOWING IN SOYBEAN

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Introduction

Viral infections have been identified as a severe biotic threat to soybean cultivation worldwide. Among the viruses infecting soybean, begomoviruses belonging to the Family Geminiviridae reported to cause detrimental effects to soybean production. In Sri Lanka too, leaf yellowing diseases cause significant economic losses to soybean cultivations. However, the records on yield losses are not available. Among begomoviruses, Horsegram yellow mosaic virus (HgYMV), a whitefly-transmitted virus was firstly reported in 2010 [1] and has been identified as a severe threat to soybean production in the country. HgYMV is reported to be infecting many legume crops grown in India since 1970s [2]. In Sri Lanka too HgYMV is a serious threat to common bean cultivations and the yield losses could reach up to 50%. Recently a weed (*Hedyotis corymbosa*) that harbors the HgYMV have also been identified [3]. Having observed the severe effects on soybean due to yellowing symptoms, and knowing that the legumes are highly affected by begomoviruses, this study was done to identify the causal virus of yellowing in soybean and to verify the transmission of the causal virus.

Materials and Methods

Sample collection and DNA extraction from leaf tissues

Soybean samples expressing characteristic yellowing symptoms were collected from experimental fields of Horticultural Crop Research and Development Institute, Sri Lanka. Total DNA was extracted by CTAB (Cetyl trimethyl ammonium bromide) extraction protocol [4] with a few modifications. A sample of 150-200 mg of tender leaf tissues were homogenized with 1.5 ml of preheated (to 65 ^{0}C) DNA extraction buffer (0.1 M Tris HCl, 0.2 M EDTA, 1.4 M NaCl, 2% CTAB, 1% PVP, β-mercaptoethanol). The homogenized mixture was transferred into a 1.5 ml Eppendorf tube and incubated at 65 ^{0}C for 30 min. Then, the tube was centrifuged at 2,000 rpm for 1 min and the supernatant was transferred into a new Eppendorf tube. An equal volume of chloroform: isoamylalcohol (24:1) was added and mixed by gentle inversion and centrifuged for 10 min at 12,000 rpm. The resulting supernatant was transferred to a fresh Eppendorf tube and added isopropanol. The tube was then inverted several times and incubated overnight at -20 ^{0}C. DNA was pelleted by centrifugation for 5 min at 12,000 rpm. The pellet was then washed with 70% ethanol and dried at room temperature for 20 min until all traces of ethanol dried. Finally the pellet was dissolved in 100 µl of 1X TE buffer and stored at -20 ^{0}C.

PCR amplification mixture (1 X)

PCRs with Deng primers (Integrated DNA Technologies, U.S.A.) were performed in a thermo-cycler, using 25 µl reaction mixtures. This mixture consisted of 2.0 µl of total DNA extracted from infected soybean leaf tissues and diluted up to 1/25 (80-100 ng), 0.2 µl Taq DNA polymerase (5U/ µl), 2.5 µl of 10X PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 0.5 µl of 25 mM $MgCl_2$, 2.0 µl each primer (10 mM), 2.0 µl dNTPs mix (2.5 mM each), and sterile water to make up the volume.

Amplification of HgYMV DNA using Deng 540 and Deng 541 primers

Degenerated primers, Deng 540/541 (forward 5'-TAA TAT TAC C(K)G(W)(K)G(V) CC(S)C-3', reverse 5' TGG AC(Y)T T(R)CA(W) GG(B)C CTT CAC A-3') [5] were used in this study to detect begomoviruses associated with soybeans at a concentration of 1/25 (DNA: sterile water). The mixture underwent one cycle of initial denaturation at 94 ^{0}C for 5 min followed by 30 cycles of denaturation at 94 ^{0}C for 30 s, annealing at 58 ^{0}C for 30 s, extension at 72 ^{0}C for 45s and a final extension at 72 ^{0}C for 10 min. The PCR products were analysed by 1% agarose gel electrophoresis at 60 V for 1 h in 1X TBE buffer [100 mM Tris (pH 8), Boric acid, 0.5 M EDTA (pH 8)] with a loading of 5.0 µl product per well. The gel was previously stained with ethidium bromide at 0.5 µg/ml. Amplification product size was estimated using 100 bp DNA ladder (Vivantis, Germany). DNA from healthy soybean plants and double distilled water were used instead of template DNA as experimental controls. Further resulted amplicon was compared with previously amplified HgYMV DNA isolated from horsegram (original host).

Sequencing of the PCR product and homology search

Fragment of coat protein gene present in genome A component of the begomoviurs was amplified using said primer pair and sent to GeneTech Pvt. Ltd., Colombo 08, Sri Lanka for bidirectional sequencing. Sequence information obtained was subjected to FASTA analysis. Sequences with FASTA forms were edited and resulted fragment was analyzed using Basic Local Alignment Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST) based on the close sequence identity and the length of the sequences. Thereafter, nucleotide sequences pertaining to coat protein regions of begomovirus were downloaded from GenBank with the accession numbers provided by the FASTA output and fed into MEGA7.0 software to determine phylogenetic and molecular evolutionary relationships by neighbor-joining method [6] at 1000 bootstrap value.

Transmission of HgYMV through whiteflies

After confirmation of the causal virus of the soybean yellowing as a begomovirus, through PCR and homology search, transmission tests were conducted. To this end, nonviruliferous whiteflies reared on brinjal plants (immune to HgYMV) were randomly collected using an aspirator and transferred for acquisition feeding for a period of 24 hours on soybeans that were confirmed to be infected by HgYMV based on PCR, DNA sequencing and homology search. After 24 hours, the , feeding of the whiteflies was interrupted by slightly shaking the plants and again transferred them

on to healthy seedlings of soybean, common bean and horsegram (approximately 10 whiteflies/ plant) inserted into mini transmission cages made out of 500 mL empty transparent polythene bottles. After a period of 12 hours the, cages were removed and the plants were sprayed with (Admire®, Bayer Crop Sci.) 200 g / L SL (1 mL/ L) and were kept for 21 days under insect proof greenhouse environment to observe possible virus like symptoms pertaining to HgYMV (e.g. mild yellowing on leaves). Back inoculation was performed from soy bean to common beans and horsegram. Accordingly, transmission ability of the causal virus from soybean to soybean, soybean to common bean, common bean to soybean, horsegram to soybean and soybean to horsegram was tested. The rate of infection in each case was calculated with the formula, [(Number of plants with virus like symptoms/ Number of plants inoculated with whiteflies) x100]. The experiments were repeated three times at a rate of 10 seedlings in each repeat. Development of symptoms characteristic to the infection, namely initial mild yellowing progressing through the entire leaf with time was observed in the plants used for virus transmission.

Results and Discussion

The results confirmed the causal virus of the yellowing in soybean is a begomovirus and can be successfully detected in infected tender soybean leaves using Deng 540/541 primers due to the resulting in amplicons of 520 bp (Figure 1). Deng 540/541 primers have been employed by a number of researchers both locally and internationally to detect begomovirus [3,7].The amplified DNA fragment of the of the present study (SbYD-Partial) showed 96% sequence homology to HgYMV-LK: 09-Bean isolate (GenBank accession number GU323321) which has been isolated from common beans in Sri Lanka [1]. SbYD-Partial (Figure 2) further showed homologies in the range of 89-91% to several HgYMV isolates reported from India in Horsegram and several other legumes including Lima bean, French bean.

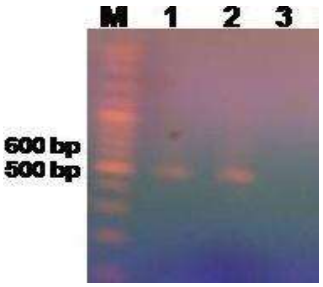
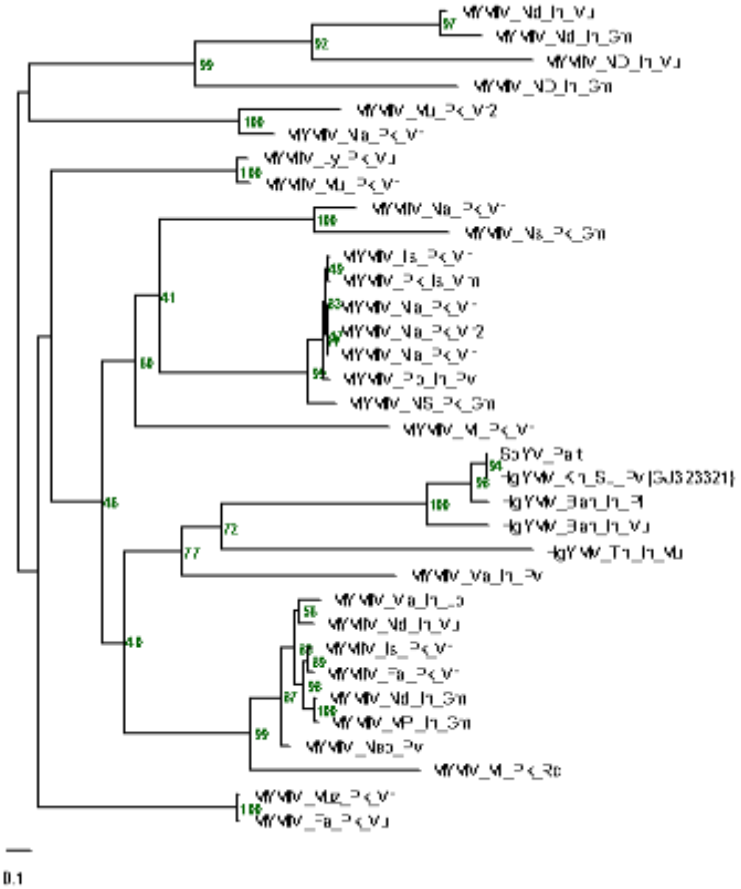


Figure1: Amplicon resulted through PCR. Lane (L) M: DNA marker 100 bp, L1:HgYMV (soybean), L2: HgYMV (control from Horsegram , L3: Healthy soybean

Figure2: Neighbor joining tree constructed using closely related begomovirus sequences



The DNA sequence of the present study formed a sub cluster with HgYMV-LK:09-Bean (GU323321) from beans in Sri Lanka, indicating the genetic similarity of SbYD-Partial with GU323321(Figure 2). Furthermore, both the sequences (SbYD-Partial and HgYMV-LK:09-Bean) formed a distinct cluster with other HgYMV isolates reported in India suggesting the close genetic relationship of SbYD-Partial with the HgYMV isolates in India.

Transmission study

The results of the transmission test revealed that the virus can be cross transmitted among the selected plants (i.e. soybean, common beans and horsegram) at varying rates through viruliferous whiteflies (Table 1). Accordingly, transmission from soybean to soybean, soy bean to common bean, common bean to soybean, horsegram to soybean and soybean to horsegram occurred. Higher transmission rates were obtained when it was transmitted from horsegram to soybean and common bean to soybeans.

Table1. Results of the transmission test using viruliferous whiteflies

From	To	Symptoms	Rate of infection
Soybean	Soybean	Mild yellowing	80%
Soybean	Common bean	Mild yellowing	40%
Common bean	Soybean	Mild yellowing	80%
Horsegram	Soybean	Mild yellowing, Downward curling	90%
Soybean	Horsegram	Mild yellowing, Downward curling	50%

Conclusions

Begomovirus causing yellowing disease in soybean can rapidly and precisely be detected by PCR with universal primers, Deng Deng 540/541. The causal virus of the disease is a variant of Horsegram yellow mosaic virus. The virus can be transmitted among soybean, common bean and horsegram, with the highest infection rate when from horsegram (HgYMV's original host) to soybean through whiteflies. In future, this information can be utilized to formulate effective integrated management programs to control the spread of HgYMV with a special emphasis on alternative hosts.

References

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