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Croton Yellow Vein Mosaic Virus Found Infecting Naturally Growing Weeds in India

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ABSTRACT

Weeds remain major problem whether they are competiting for the nutrition or they are acting as a reservoir host of many plant viruses. During survey of Delhi NCR region in India, many weed plants were collected on the basis of symptoms from the vicinity of the crop fields. Weeds checked for virus presence were : Ageratum, Verbesina, Amaranthus, *Solanum nigrum*, Croton, Calotropis, *Datura metel* and Congress grass. Initial detection was done using PCR using primers for CP region which was followed by cloning and sequencing. Major virus which was found in three weed plants (Verbesina, Ageratum and Amaranthus) was Croton yellow vein mosaic virus. Out of eight weed plants tested for begomoviruses, three were found positive.

Key words : Weeds, amaranthus, verbesina, ageratum, begomovirus

INTRODUCTION

Begomovirus genera of Geminiviridae family is characterized by twin geminate icosahedral particle morphology. Geminiviruses have been classified to nine genera based on the characteristic features like host range, vector, organization of the genome viz., *Becurtovirus, Begomovirus, Curtovirus, Eragrovirus, Mastrevirus, Topocuvirus, Turncurtovirus, Capulavirus* and *Grablovirus* (Brown *et al.,* 2015; Varsani *et al.,* 2017,). Begomoviruses are plant viruses possessing broad host range infecting dicotyledonous plants. Begomoviruses are sub-divided into two different classes based on geographical origin old world and new world viruses which are of either bipartite or monopartite and bipartite, respectively (Varma *et al.*, 2011, 2012, 2013).

Begomoviruses, which are mostly native to the new world, have genomes comprising two components of ssDNA of which are of ~2800 nt and are known as DNA A and DNA B and from various studies it is evident that both the components are required for the systemic infection of plants. The DNA A encodes all viral proteins which are required for the replication of viral DNA, for gene expression and transmission to other plants, while the DNA B component encodes only two proteins which are essential for inter- and intracellular movement of virus in the host plants. Some bipartite begomoviruses also occur in the old world, but mostly these are monopartite, their genomes have only DNA A which is similar to DNA A component of bipartite viruses (Chandel *et al.,* 2016).

Begomoviruses (Geminiviridae) are largest group of viruses infecting all kinds of plants such as ornamental, pulses, vegetables and also wide range of plants which come under non-cultivated category such as weeds belonging to different plant families. Weeds have always been acting as reservoirs of not only begomoviruse/s but also other plant pathogens such as fungi and bacteria. As number of begomoviruses/viruses are infecting a single weed plant. This also leads to recombination and thus new virus strains and species. Weeds acting as reservoirs can play an important part in the emergence of plant viral epidemics affecting crops. Due to the importance of weed hosts in the epidemiology and evolution of begomoviruses it becomes thus important to study viruses that infect these plants. Begomoviruses have emerged as a major constraint in a large number of crops throughout the tropical and sub-tropical world (Varma *et al.*, 2011). India is one of the important centers of diverse begomoviruses infecting a wide range of crops and weeds. Weeds were earlier looked upon as plants which steal the nutrition of crops but major studies have been carried out in the past which reveal the truth that these are actually alternate host to several important plant viruses. Survey at

different places in Delhi NCR region for weeds growing in the vicinity of the crop fields, typical begomovirus like symptoms were observed.

Weeds such as Ageratum, Verbesina, Amaranthus, *Solanum nigrum*, Calotropis, Datura and Croton, are found very commonly in India growing in barren fields, wastelands, alongside roads and also along with crop fields. Weeds are known to compete with crops for nutrition and space but besides this these are alternate and reservoir hosts of many pathogens including viruses. When survey was conducted in the NCR, many weed plants were seen displaying viral symptoms. Diagnosis by PCR confirmed the virus infection.

Number of viruses have been reported from weed plants from all over the world. Tobacco curly shoot virus infecting wild sunflower (Accession HQ407395), partial sequence data on *Ageratum enation virus* (AEV; Verbesina encelioides yellow vein Lakshmangarh virus) isolate (JN998449) and Ageratum leaf curl betasatellite (Verbesina yellow vein betasatellite) isolate (JQ693145) infecting wild sunflower (*Verbesina encelioides*) are reported from India. Deinbollia mosaic virus was reported from Deinbollia in Tanzania by Kyallo *et al.* (2017). Ageratum enation virus is also infecting amaranthus as reported from India by Raj *et al.* (2008). Tomato yellow leaf curl virus was detected by using nucleic acid tissue blot hybridization in Amaranthus sp. (Abou-Jawdah *et al.,* 1999). Begomoviruses were also reported on two species of Amaranthus, *Amaranthus spinosus* and *A. viridis* (Arnaud *et al.,* 2007). Many Amaranthus species were reported as the natural hosts of many viruses belonging to different groups (Horvath, 1991; Raj *et al.,* 1997). Ageratum enation virus was reported from *Ageratum conyzoides* by Tahir *et al.* (2015). On Sonchus Alternanthera yellow vein virus was reported from Pakistan by Mubin *et al.* (2010). From croton also, Croton yellow vein virus was reported from Pakistan by Hussain *et al.* (2011).

MATERIALS AND METHODS

Weed plants viz., Ageratum, Verbesina, Amaranthus, *Solanum nigrum* and Croton, showing typical symptoms of begomovirus infection (yellow mosaic, leaf curling, smaller leaflet and stunting) were collected from various fields (chilli, and tomato). Some weeds not displaying any symptoms such as Calotropis, Datura, Congress grass were collected from Noida, India. Samples were taken with purposive random sampling according to the typical begomovirus symptom (Fig. 1). Samples were placed in plastic bags and carried to the laboratory for DNA extraction.

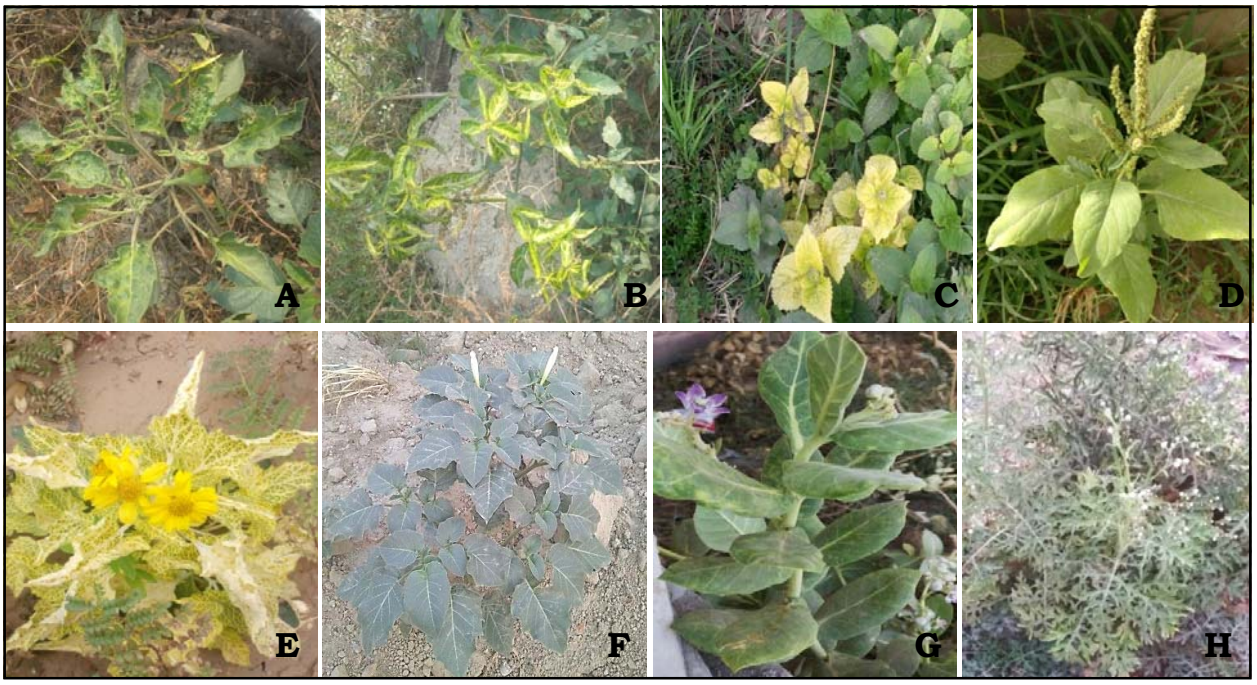

Fig. 1. Weed plants displaying symptoms of virus infection.
A. *Solanum nigrum,* B. Croton, C. Ageratum, D. Amaranthus, E. Wild sunflower (Verbesina), F. *Datura metel,* G. Calotropis and H. Congress Grass.

DNA was extracted from infected leaves of Ageratum, Verbesina, Amaranthus, *Solanum nigrum*, Croton, Calotropis, Datura and Congress grass using CTAB method (Permingeat *et al.,* 1998) following standard protocol. About 200 mg of leaf sample was used for DNA extraction. Leaf samples were crushed in CTAB buffer and incubated for 20 min at 55°C in a water bath. Chloroform : Iso Amyl Alcohol (24 : 1) was used for extraction of DNA. Final precipitation was done by ammonium acetate followed by ice cold ethanol. Purity of DNA was checked by agarose gel electrophoresis. The primer pair AV494 and AC1048 was used to amplify the partial coat protein gene AV1 and 550bp amplification was expected. Each reaction contained 1µl DNA, 1 unit Taq Polymerase (Invitrogen), 0.3 µl dNTP mix, 0.3 µl of each primer (10 mM), 2.5 µl 10x Taq Buffer (*In vitro* gen) and sterile water to make up total volume of 25 µl. Initial denaturation was done at 94°C for 2 min and then thermal-cycler (BioRad) for 34 cycles : denaturation at 94°C for 30 sec, annealing temperature was 55°C for 30 sec and elongation at 72°C for 90 sec. Final extension was done for 10 min at 72°C. PCR amplified amplicons were screened by using agarose gel electrophoresis and was stained using EtBr. For betasatellite primers given by Briddon *et al.* (2002) were used. For alphasatellite primers by Singh *et al.* (2011) were used. Amplified products were cloned in TA cloning vector (Promega) and were sequenced (Table 1).

Table 1. Primers used in the study

Primer name	Primer sequence
AC 1048 f	GGRTTDGARGCATGHGTACAT
AV 494 r	GCCYATRTAYAGRAAGCCMAG
Beta 01	5'GGTACCACTACGCTACGCAGCAGCC3'
Beta 02	5'" GGTACCTACCCTCCCAGGGGTACAC3'

RESULTS AND DISCUSSION

Good quality DNA was obtained by CTAB and was used in PCR. Amplification of 550bp was obtained in PCR (Fig. 2) by Wyatt primers, which confirmed the possibility of a begomovirus infection in the samples of Ageratum, Verbisina, Croton and Amaranthus. After cloning and sequencing BLASTn analysis was done and Croton yellow vein mosaic virus was found in Ageratum and Verbisina and Amaranthus. However, in croton, no significant

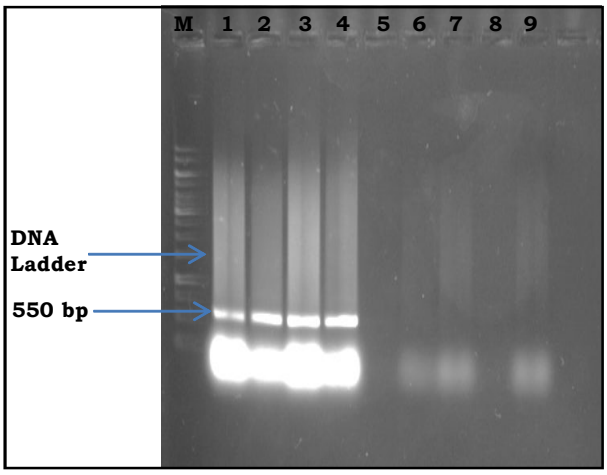

Fig. 2. PCR amplification in Lane 1–Verbesina, Lane 2–Amaranthus, Lane 3–Croton, Lane 4–Ageratum 550bp and Lane M–DNA Ladder.

results were obtained. Virus on Ageratum (Acc No. MH720957) was found to be matching 98% with Croton yellow vein mosaic virus. CYVMV on verbesina (Acc. No. MH720958) showed 91% similarity to CYVMV infecting Croton and reported from Pakistan and CYVMV on Amaranthus (Acc. No. MH720959) showed 98% identity to CYVMV earlier reported from Croton. Possibility of same virus on the three weeds is because weeds were collected from similar place. Papaya leaf curl betasatellite was found associated with CYVMV infecting Verbesina, whereas no betasatellite was found with other viruses. We did not find any alphasatellite molecule with any virus. However, Amaranthus and Verbisina are new hosts of this virus. Begomovirus infection was also reported in many plants where no typical symptoms of virus infection were visible (Tavares *et al.*, 2012). Along with plants showing symptoms some plants such as Calotropis, Datura, Amaranthus and Congress grass were also taken for virus detection. But no amplification of virus was found in these plants except Amaranthus. Some plants showed symptoms but still we could not detect any virus such as Croton which showed typical symptom of vein clearing, but no virus was detected.

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