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MOLECULAR CHARACTERIZATION OF *BEGOMOVIRUS* INFECTING *ABUTILON GLAUCUM* IN SOUTH GUJARAT REGION

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ABSTRACT
Weeds are potential sources of primary inoculum of viruses and play an important role in their persistence and spread. During the rainy season of 2010 - 2012, several plants of *Abutilon glaucum* in and around Navsari Agricultural university have been found with vein yellowing symptoms. In severe cases, the chlorosis extends into interveinal areas, resulting in complete yellowing of the leaves. Leaf samples from different plants showing severe symptoms were collected and tested for the presence of begomoviruses using PCR assay with the sets of degenerate primers designed to amplify part of the DNA-A strand. A PCR product of the expected sizes (750bp) was obtained with all infected samples. A 696 bp sequence of DNA-A molecule was obtained and submitted to GenBank (Accession Nos. JX258326). The highest nucleotide identity of 93% (BLASTn, NCBI) was obtained with Kenaf leaf curl virus-[India:Kaisarganj:2007]. The phylogenetic reconstruction were done by comparing sequence with other *begomovirus* AV1 gene. A survey of literature revealed that there is no record of a viral affecting this weed and, therefore, this is the first report of a viral disease affecting this weed plant.

INTRODUCTION

Geminiviruses are divided into four genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Begomovirus* based on genome organization, host range, and insect vector (Fauquet *et al*., 2008). Geminiviruses belonging to the genus *Begomovirus*, are exclusively transmitted by the insect, whitefly (*Bemisia tabaci* Gennadius) and infect dicotyledonous plants only. *Begomovirus* is the largest genus consisting more than 180 species and several unassigned isolates. Begomoviruses inflict significant economic yield losses to many crops in tropical and subtropical regions of the world, consequent to the worldwide increase in the population and distribution of the whitefly vector and global movement of plant materials (Rojas *et al*., 2005; Seal *et al*., 2006, Mahatma *et al*., 2007). *Abutilon glaucum* belonging to the family *Malvaceae* is an annual rainy season weed of road side and fallow land found throughout the India (Hallan *et al*., 1998). The weed is commonly known as *atibala* and is being used in ayurvedic medicine (Nanda *et al*., 2012). During 2010 few plants of the weed near to Navsari Agricultural University (Navsari) were found showing yellowing of veins. Subsequent the plant started showing more conspicuous yellow colour symptoms and become complete yellow. Whiteflies were found around the weed. Through symptoms it was identified as *Begomovirus*. Many Begomoviruses have been reported to infect weeds including Sida yellow mottle virus (SiYMoV), infecting *Sida rhombifolia* plants of the family *Malvaceae* (Fiallo-Olive *et al*., 2012) and Datura leaf distortion virus" (DLDV), isolated from a *Datura stramonium* L. (Fiallo-Olive *et al*., 2013). Interestingly cross infectivity of the *Begomovirus* have been report in many crop. Recently Tomato leaf curl Bangladesh virus (ToLCBDV-Gaill) was found infecting *Gaillardia pulchella* (Mahatma and Mahatma, 2012). Under the circumstances these weeds can serve as potential source of primary inoculums and survival of the virus in off seasons. This increases the chances of epiphytotic of the diseases making disease management difficult. Therefore, a systemic investigation was carried out to ascertain cause of the disease and characterize the virus.

MATERIALS AND METHODS

Virus sources

Naturally infected *A. glaucum* plants with different types of symptoms were collected for the study of symptoms and molecular analysis.

DNA extraction

For DNA extraction, fresh leaf sample were taken and homogenized in liquid nitrogen. After complete homogenization , 4.5mL of extraction buffer (100mM Tris, pH 8.0; 100mM EDTA; 1.5 M NaCl; 1% CTAB) was added to the samples, which were then incubated for 1 h at 65ºC. DNA was extracted and purified according to method described by Sambrook and Russel (2001). RNA was removed by RNase

treatment. DNA was quantified using Nano spectrophotometer and diluted to 50ng/μL for PCR amplification.

PCR conditions and electrophoresis

PCR (Polymerase chain reaction) for specific band of DNA-A genome analysis was performed in 20μL volume containing 1x PCR buffer (Banglore Genei), 1.5mM $MgCl_2$, 0.2mM $dNTP_S$, 0.1μM of primer, 50ng of genomic DNA and 1 U Taq DNA polymerase(Banglore Genei). The reaction mixture was placed on DNA thermal cycler (Biometra). The program was performed as 1 cycle of 94ºC for 5 min and 40 cycles of 94ºC for 1min, 54ºC for 1min and 72ºC for 2min, then, a final extension step 72ºC for 8min. The specific Forward primer as LMF (CGCGAATTCGACTGGACCTTACATGGNCCTTCAC) and reverse primer as LMR (GAGTCTAGAGGATANGTRA GGAAATARTTCTTGGC) are designed from conserved part of DNA-A genome of *begomovirus* (IDT integrated DNA Technologies Coralville, IA). PCR products were electrophoresed on 1.5% (w/v) agarose gels, in 0.5X TBE Buffer at 80 V for 1h and then stained with ethidium bromide (0.5μg/mL). Gels with amplified fragments were visualized and photographed under UV light.

Sequencing of amplified PCR product

Amplified PCR product was purified using BigDye® Terminator v3.1 Cycle Sequencing clean up method described in Sambrook and Russel (2001) for sequencing. Purified product was used for Sequencing by Ready Reaction Kits (Applied Biosystem) using ABI Prism3130 automatic sequencer (Applied Biosystems, U.S.A) with same reverse and forward primers. The derived sequence was analyzed by performing online BLAST sequence homology test.

Sequence analysis

Phylogenetic trees were constructed using full optimal alignment in the Clustal_X version 1.83 Software (Thompson *et al*., 1997) and neighbor-joining method with 1000 bootstrap replications available in the MEGA version 4.0 (Tamura *et al*., 2007). Geminivirus sequences presented in Table 1 were used for the analysis.

RESULTS AND DISCUSSION

Initially around 10 plants from the area having more than 500 weeds were found showing typical yellow vein symptoms in 2010. The area was monitored constantly for the development of new symptoms and spread the disease. Spread of the disease

Table 1: Different *Geminivirus* Av1 sequences were used for comparisons and phylogenetic analyses with Kenaf leaf curl virus-Av1 gene (Abutilon glaucum: Navsari)

Virus names	Abbreviations	Accession nos.
Kenaf leaf curl virus-BALC	India:Bhangha:2007	EU822322
Kenaf leaf curl virus-KJLC	India:Kaisarganj:2007	EU822321
Kenaf leaf curl virus- BHLC	India:Bahraich:2007	EU366903
Sida yellow vein virus	Barrackpore	EU184016
Malachra yellow vein mosaic virus	-	EU285589
Kenaf leaf curl virus	LCCP-NI-Bah-02	EF620563
Kenaf leaf curl virus-Av1 gene	Abutilon glaucum:Navsari	JX258326

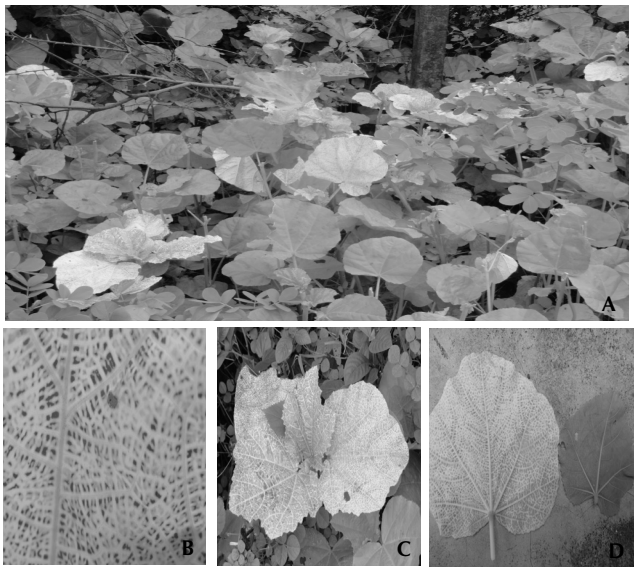

Figure 1: Symptoms of Kenaf leaf curl virus disease observed on weed *Abutilon glaucum* in open fields near Navsari Agricultural University(a: Complete view of area where disease was observed, b: Closeup view of severe symptom on leaf, Dwarfing and severe symptoms on plant, c: Severe symptoms on leaf, d: severe infection on whole plant)

was not observed in the year however, symptoms on the infected plants become more severe. Similar type of the symptoms could observed in the subsequent year also at the same place, however, diseases incidence have increased to 2 per cent. Disease could be observed around 1km away on the road side on few plants. Similar type of the disease at the same place with almost same intensity was observed in 2012 also. *Abutilon* sp. is a season specific weed propagating vegetatively in nature. Appearance of the disease at the same place again in the subsequent year might be due to survival of the virus in the vegetative part of the plant and appearance along with the development of the weed in the season. The disease is transmitted by whitefly and the disease might have spread in

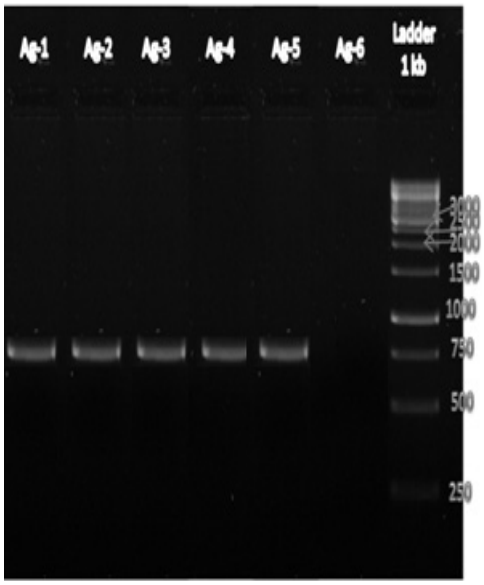

Figure 2: Positive PCR result from infected leaf

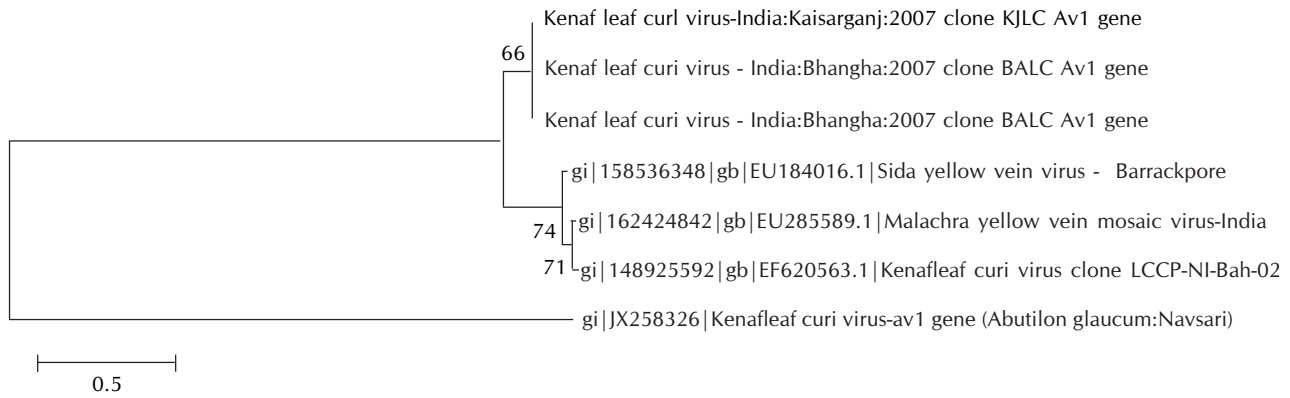

Figure 3: Phylogenetic tree based on nucleotide sequence of AV1 gene of all *Begomovirus* with *Kenaf leaf curl virus* (Abutilon glaucum:Navsari) giving similarity greater than 89% by the neighbor joining method with 1000 bootstap

another part of the area through either whitefly or vegetative part of the infected plant (Mahatma *et al*. 2012). The disease initially produced typically yellowing in few veins. Gradually symptoms become more severe covering all the veins of the leaf (Fig.1). In extreme cases, entire leaflets showed severe yellowing. Similar types of symptoms were observed in artificially inoculated plants. Samples from the plants showing different types of symptoms were collected (Ag-1 to Ag-6) and PCR was performed by using *Begomovirus* specific primers. Partial DNA-A fragments of 750 bp (Fig. 2) was amplified from all samples (Ag1-Ag5) showing typical symptoms whereas no amplification was observed from the healthy plant sample (Ag6). On sequencing, a 696 bp long sequence of DNA-A of the *Begomovirus* corresponding to the AV-1 gene was obtained and submitted to GeneBank (Accession no. *JX258326)*. Amplified sequence has maximum 93 per cent sequence identity with Kenaf leaf curl virus-[India:Kaisarganj:2007] in pairwise sequence comparison analysis, Accordingly the strain was named as *Kenaf leaf curl virus* AV1 coat protein gene. In Phylogentic analysis, three different cluster were formed (Fig. 3). First cluster include *Kenaf leaf curl virus*-KJLC (India:Kaisarganj: 2007), *Kenaf leaf curl virus*-BALC(India:Bhangha:2007) & *Kenaf leaf curl virus*- BHLC (India:Bahraich: 2007). Second cluster include *Sida yellow vein virus* – (Barrackpore), *Malachra yellow vein mosaic virus* and *Kenaf leaf curl virus* (LCCP-NI-Bah-02). While third cluster was distinctly which include Kenaf leaf curl virus (Abutilon glaucum:Navsari). Study indicated the *Kenaf leaf curl virus* observed from the Navsari is entirely distinct virus from the different viruses have been reported from the different areas from kenaf which shared maximum nucleic acid identity. Kenaf is an important fibre crop, however, is not cultivated as an important crop in Navsari area. Virus observed in the abutilon might introduce form kenaf through its vector or might have evolved in the area to infect the crop from other *Begomovirus* abundantly found in the area.

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