First Report of Begomovirus Infecting *Sonchus asper* in India

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ABSTRACT

Presence of begomovirus infection was tested through Polymerase Chain Reaction (PCR) with begomovirus-specific degenerate primer. PCR product of ~550 bp was cloned and sequenced and has been deposited in NCBI GenBank Accession No. JN000703. BLASTn analysis was conducted with other begomovirus isolates in GenBank database at NCBI. The coat protein sequence of the virus associated with yellow mosaic disease of *S. asper* revealed the highest 98% nucleotide sequence identities with Ageratum enation virus - Gorakhpur AV1 gene (EU867514) and 97% with both Ageratum enation virus isolate Lucknow coat protein (AV1) gene (JF682242) and Ageratum enation virus isolate Gorakhpur (GQ268327). To our best knowledge, this is the first report of Begomovirus associated with yellow mosaic disease of *S. asper* from India.

Key words: *Sonchus asper*, Begomovirus, PCR, BLASTn

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INTRODUCTION

Begomoviruses may infect weeds, ornamentals etc and they may serve as a reservoir for crop-infecting geminiviruses¹. *S. asper* (family *Asteraceae*) is an annual edible weed plant with spiny leaves and yellow flowers found in India annually. The leaves are lanceolate, light green, with wavy margins covered in spines on both the margins and beneath. The leaves and stems emit a milky sap when cut. The flowers grow in clusters and the end of the stems. It is used as edible leaves make a palatable and nutritious leaf vegetable.

Begomovirus is one of the largest genera of the family *Geminiviridae*^{2,3} and its carrier white fly (*Bemisia tabaci*) prevails more in the tropical and subtropical regions of the world⁴. It has a bipartite genome having two circular DNA designated as DNA-A and DNA-B whose size ranges from 2.7 to 3.0 kb. An additional circular DNA has been reported as β satellite which contributes to *Begomovirus* virulence⁵. The study was undertaken to find out the infection of begomovirus in *S. asper* by using molecular diagnostic techniques.

MATERIALS AND METHODS

Infected leaf samples of *S. asper* were collected from the fields of Lakshmangarh, Rajasthan (India). The DNA was extracted from infected leaves using CTAB method⁶ and PCR was carried out using universal coat protein primer for begomovirus⁷. The sequence of forward

primer was GGRTTDGARGCATGHGTACATG (AC 1048) and sequence of reverse primer was GCCYATRTAYAGRAAGCCMAG (AV 494). The PCR product was cloned, partially sequenced and submitted in NCBI. Homology sequence search was carried out by using BLASTn. Phylogenetic tree was constructed by using MEGA 4.0⁸. In order to confirm the geminivirus infectivity Southern blotting was performed according to the method of Kon⁹. Cloned DNA-A of Ageratum enation virus was used to develop the radio-labeled probes.

RESULTS AND DISCUSSION

During December 2010 an extensive survey for whitefly-transmitted geminiviruses, symptoms such as yellow mosaics, leaf curling and decrease in leaf size were observed (Fig. 1) in *Sonchus asper* plants (family

Fig. 1: Typical begomovirus infection symptoms in weed *Sonchus asper*

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Fig. 2: PCR amplification of begomovirus isolated from weed *Sonchus asper* collected from Lakshmanagarh, Rajasthan (India). 1% agarose gel of PCR amplification products obtained with universal coat protein primer. DNA used in the PCR from the host *Sonchus asper* [lane 1 to 4]. The approximately 550 bp bands in lane 1 to 4 represent the amplified products from infected host plant DNA. L1 is 100 bp middle range DNA Ladder (Bangalore Genei, India)

Fig. 3: Neighbor-Joining tree based on the *Sonchus* yellow mosaic virus [coat protein gene, JN000703] isolated from *S. asper* and other begomovirus sequences available in GenBank. (FR837932) Cotton leaf curl Burewala virus complete genome, clone MV13. (AM774295) Cotton leaf curl virus-[Burewala] DNA A, complete sequence, clone LA Kw5. (FN552005) Cotton leaf curl Shadadpur virus, complete genome, isolate LS6. (GU385766) Cotton leaf curl virus isolate Lucknow movement/precoat protein (AV2) gene, partial cds; coat protein (AV1) gene, complete cds. (HQ257375) Radish leaf curl virus isolate IN: Bih06:10 segment DNA-A, complete sequence. (EU194914) Euphorbia leaf curl virus isolate Pusa Bihar, complete genome. (AF240675) Tobacco curly shoot virus-[Y1] DNA A, complete sequence. (EF119337) Crotalaria juncea begomovirus coat protein (CP) gene, complete cds. (FJ455449) Sunn hemp leaf distortion virus clone SHLD-FL-Bar-01 segment DNA-A, complete sequence. (DQ116879) Chili leaf curl Pakistan virus isolate Khanewal 2 clone PC5 segment A, complete sequence. (DQ114477) Chilli leaf curl virus-[Pakistan] segment DNA A, complete sequence. (EU867514) Ageratum enation virus-Gorakhpur AV1 gene, complete cds. (GQ268327) Ageratum enation virus isolate Gorakhpur, complete genome. (JF682242) Ageratum enation virus isolate Lucknow coat protein (AV1) gene, complete cds. (GU199583) Tobacco curly shoot virus clone 20-9, complete genome. (AM701770) Ageratum enation virus AV1 gene, AV2 gene, AC1 gene, AC3 gene, AC2 gene and AC4 gene clone Turnip A. (AJ810825) Ageratum yellow vein virus-Pakistan V2 gene for coat protein. (AM698011) Ageratum enation virus, complete genome. (AM261836) Ageratum enation virus complete genome, clone SonAL8 (3)

Asteraceae) growing as common weed in the fields of Sikar, Rajasthan, India. Symptomatic leaf samples were collected. To investigate the possibility of geminivirus, total genomic DNA was extracted from leaves tissues of *S. asper* plant infected with yellow mosaic disease using CTAB method.

PCR product (Fig. 2) of ~550 bp was sequenced and submitted in NCBI (GenBank accession JN000703). Sequence analysis of JN000703 (*Sonchus* yellow mosaic virus isolate MP 2 coat protein gene) was done by using BLASTn revealed the highest 98% nucleotide sequence identities with *Ageratum* enation virus-Gorakhpur AV1 gene (EU867514) and 97% with both *Ageratum* enation virus isolate Lucknow coat protein (AV1) gene (JF682242) and with *Ageratum* enation virus isolate Gorakhpur (GQ268327). Hybridization of *Ageratum* enation virus probe with the DNA fragment on the filter membrane further indicates that this fragment contains DNA sequence that is complementary to the probe. The strong signal showed that the virus in *S. asper* had some homology with the *Ageratum* enation virus.

Phylogenetic analysis was done by using MEGA 4.0 showing the relationship with other closely related viruses. In Neighbor-Joining tree, the coat protein gene of *Sonchus* yellow mosaic virus place in a separate monophyletic cluster of 58 bootstrap values with JF682242, GQ268327 and EU867514 [82 bootstrap] (Fig. 3), therefore these results indicating that it is a new Begomovirus isolate infecting *S. asper*.

Future studies will be focused on the amplification of the complete genome of the viruses. Moreover a recombination analysis and a detailed survey of its distribution and host range in distinct locations of the country will be carried out. The possible association of a Begomovirus with *S. asper* had not been tested previously, so this is the first ever report of Begomovirus infecting economically important noxious weed *S. asper*. Therefore *Sonchus* yellow mosaic virus is a new reported species as a distinct begomovirus infecting *S. asper*. Thus, identification of a Begomovirus represents the possibility of a serious threat to other economically important ornamental and crop plants.

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REFERENCES

1. Roye, M.E., W.A. McLaughlin, M.K. Nakhla and D.P. Maxwell, 1997. Genetic diversity among geminiviruses associated with the weed species *Sida* sp., *Macroptilium lathyroides* and *Wissadula amplissima* from Jamaica. *Plant Dis.*, 81: 1251-1258.
2. Fauquet, C.M., M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball, 2005. *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*. Elsevier Academic Press, San Diego, California, USA.
3. Marwal, A., A. Sahu, R. Prajapat, D.K. Choudhary, R.K. Gaur, 2012. First report of association of a begomovirus with the leaf curl disease of a common weed, *Datura innoxia*. *Indian J. Virol.*, 23: 83-84.
4. Markham, P.G., S.L. Bedford and M.S. Pinner, 1994. The transmission of geminivirus by *Bemisia tabaci*. *Pesticide Sci.*, 42: 123-128.
5. Briddon, R.W., S. Mansoor, I.D. Bedford, M.S. Pinner and K. Saunders *et al.*, 2001. Identification of DNA components required for induction of cotton leaf curl disease. *Virology*, 285: 234-243.
6. Sharma, A.D., P.K. Gill and P. Singh, 2002. DNA isolation from dry and fresh samples of polysaccharide-rich plants. *Plant Mol. Biol. Rep.*, 20: 415-415.
7. Hallan, V., 1998. Genomic organization of geminivirus causing leaf curl in tomato (*Lycopersicon esculentum*). Ph.D Thesis. University of Lucknow, Lucknow, India.
8. Prajapat, R., A. Marwal, A.K. Sahu and R.K. Gaur, 2012. Molecular *in silico* Structure and Recombination Analysis of Betasatellite in *Calotropis procera* Associated with Begomovirus. *Arch. Phytopathology Plant Prot.*, 45: 1980-1990.
9. Kon, T., L.M. Dolores, N.B. Bajet, S. Hase, H. Takahashi and M. Ikegami, 2003. Molecular characterization of a strain of Squash leaf curl China virus from the Philippines. *J. Phytopathol.*, 151: 535-539.