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Evidence of the Association of *Solanum leaf curl lakshmangarh virus* with a Weed Plant *Solanum nigrum* in Rajasthan, India

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ABSTRACT

Background: Over the past few decades, there has been more interest in geminiviruses, especially begomoviruses, as many of the diseases they cause have now reached epidemic proportions. Weed plants serve as an alternative host for begomoviruses and may allow the transmission of begomovirus to other horticulture crops and medicinal plants thus enhances the host range of these viruses in different regions of the India. The main aim of the study is the identification and molecular characterization of a begomovirus infecting a weed plant *Solanum nigrum*. **Methods:** DNA was extracted and subjected to Polymerase Chain Reaction. Positive samples were cloned and sequenced and nucleotide identity was revealed. Further phylogenetic analysis was carried out to trace the evolutionary history of the identified begomovirus. **Results:** A begomovirus was found associated with the disease. The associated begomovirus was identified as a *Solanum Leaf Curl Lakshmangarh Virus* (SoLCLV) based on nucleotide sequence of the viral genome (JN009667). Sequence analysis of JN009667 was done by using BLASTn revealed the highest 96% sequence identity with *Cucurbita pepo begomovirus* [Gorakhpur coat protein, EU366164]. **Conclusion:** A new disease of *Solanum nigrum* with characteristic mild leaf curl and yellowing symptoms was observed in 2010 in plants grown as common herb in the fields of Lakshmangarh, Rajasthan, India. This is the first report of yellow leaf curl disease of *Solanum nigrum* in Rajasthan province of India.

Key words: Weed, begomovirus, *Solanum nigrum*, SoLCLV, India

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INTRODUCTION

Geminiviruses were recognized by the International Committee on the Taxonomy of viruses (ICTV) on the basis of their unique virion morphology and possession of ssDNA as their genomic material in 1978[1,2]. Geminiviruses serve as a major plant pathogens in tropical and subtropical countries[3,4,5] affecting higher range of crops, weed and other plants that cause disastrous impact on productivity. The family Geminiviridae members have a circular, single-stranded DNA (ssDNA) genome, approximately 2.7-5.2 kb. Based on their genome arrangement and biological properties, geminiviruses are classified into four genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus*[6].

A weed common to Puerto Rico was found to be infected with *Bean Golden Mosaic Virus* (BGMV) which caused bright yellow mosaic symptoms in *Macroptilium lathyroides*,[7]. For *Tomato Yellow Leaf Curl Virus* (TYLCV), weeds act as a reservoir or 'Transmission Bridge' between cropping and non-cropping seasons[8]. Tobacco, papaya, tomato, *Ageratum conyzoides*, *Alternanthera philoxeroides* and *Sida acuta* are the different crops and weed hosts which were found to be infecteed with many begomoviruses as reported from China[9]. The report of begomoviruses in *L. hyssopifolia* weed from China suggests that *L. hyssopifolia* was an adaptive host for begomoviruses[10].

Solanum nigrum (Black night shade) is one of the medicinal herbs or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats that belong to the family *Solanaceae*. The *Solanum nigrum* is worldwide distributed weed and, it is native to Eurasia, introduced in the Americas, Australia and South Africa. The medicinal values of the *Solanum nigrum* has been mentioned in ancient literature as useful in disorders of inflammation. Juice of fresh *Solanum nigrum* herb used to treat inflammation[11], edema[12], mastitis and hepatic cancer for a long time in oriental medicine. The study was undertaken to find out the infection of begomovirus in *Solanum nigrum* by using molecular diagnostic techniques.

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MATERIALS AND METHODS

Extraction of total DNA: The leaf samples were cleaned, cut, rolled in a piece of tissue paper and was stored at-20°C until DNA isolation. To begin with the molecular characterization total DNA was extracted from leaves of infected as well as healthy weeds using the Cetyl Trimethyl Ammonium Bromide (CTAB) method[13]. Samples of the total DNA product was analyzed by electrophoresis on a 1-2% agarose gel and the concentration was determined by using Nanodrop (Thermo Scientific).

Identification of begomovirus components by PCR: A typical PCR reaction contained about 2.5 μL DNA template, 2.5 μL*Taq* 10×buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl 1% [v/v]) 1.5 μL $MgCl_2$, 2 μL of dNTP mix, 0.5 μL of *Taq* DNA polymerase and 1 μL of each primer. The PCR reaction mixture was prepared in 14 μL of RNAase free water. The PCR thermal profile for AC-1048 and AV-494 primer[14] set were pre-PCR denaturation at 94°C for 2 min followed by 35 cycles of denaturing at 94°C for 60 sec, annealing at 55°C for 45 sec and extension at 72°C for 45 sec and a final extension at 72°C for 5 min. The amplicons were analyzed by electrophoresis using 1-2% (w/v) agarose gel in TAE buffer (40 mMTris-acetate, 1 mM EDTA pH 8.1).[15].

Cloning, sequencing, phylogenetic analysis and southern blotting: The PCR products that were showed amplification up to the expected size were cloned and sequenced (Gen Bank Accession number JN009667). Sequence analysis was done by using BLASTn. Phylogenetic tree was constructed by using MEGA 4.0[16]. In order to confirm the geminivirus infectivity Southern blotting was performed according to the method of[17]. Cloned DNA-A of Ageratum enation virus was used to develop the radio-labeled probes.

RESULTS AND DISCUSSION

Results: During a survey of fields in Lakshmangarh, Rajasthan, India, in 2010 begomovirus associated symptoms were observed such as curling of leaf, yellow vein mosaic, decrease leaf size and stunting (Fig. 1) in *Solanum nigrum* plants grown as common herb in the fields of Lakshmangarh, Rajasthan, India. Among the major diseases of *S. nigrum*, the leaf curl disease is the more serious problem with economic implications. Such infections were known to reason due to observed population of *Bemisia tabaci* near infected plants.

PCR products were showed amplification of a product of the expected size (~550 bp) during agarose gel electrophoresis only in infected samples not in healthy samples. The PCR product of expected size ~550 bp was sequenced and deposited in NCBI (Accession number JN009667) as *Solanum leaf curl Lakshmangarh virus* [clone AV1 Coat Protein isolate-RaAv 04].

Sequence analysis of JN009667 was done by using BLASTn revealed the highest 96% sequence identity with *Cucurbita pepo begomovirus* [Gorakhpur coat protein, EU366164] and 95% sequence identities with both *Tomato leaf curl Palampur virus* [isolate ToLCPMV: Var:

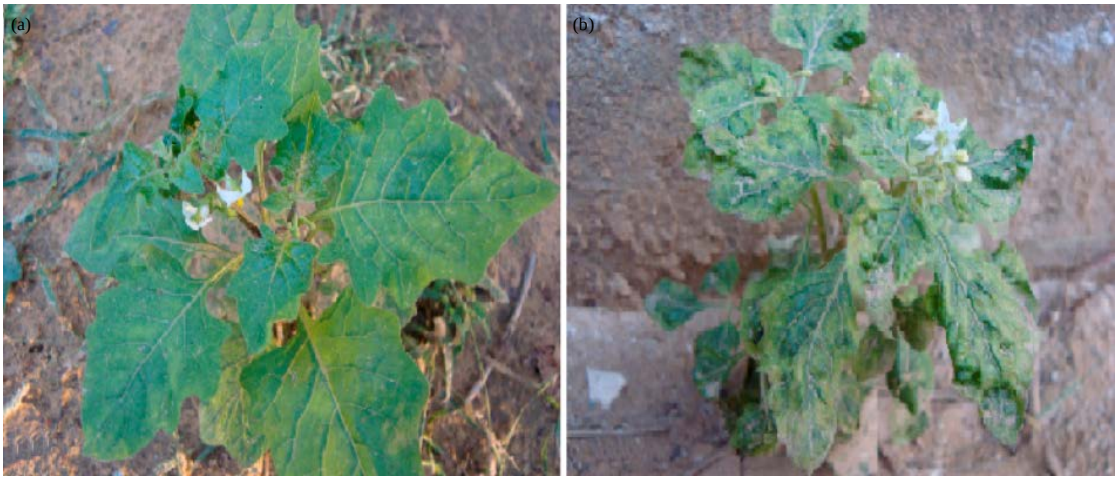

Fig. 1(a-b): (a) Healthy weed plants of *Solanum nigrum* collected from the fields of Lakshmangarh, Rajasthan, India and (b) Natural begomovirus infection in *Solanum nigrum* exhibiting yellow leaf curl disease in leaf resulting in crinkled leaves and stunted growth

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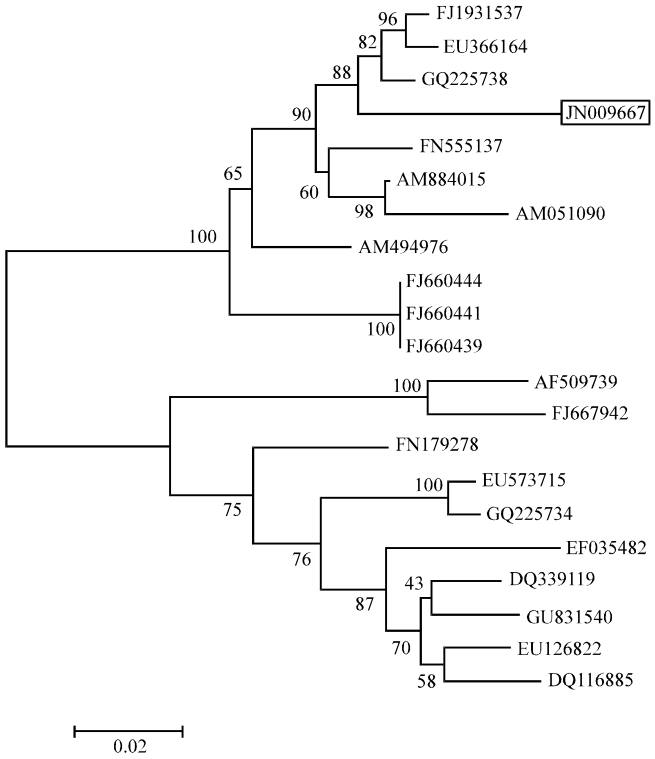

Fig. 2: Neighbor-Joining tree based on *Solanum leaf curl Lakshmangarh virus* [clone AV1 Coat Protein isolate-RaAv 04] isolated from *Solanum nigrum* and other begomovirus sequences available in GenBank. Bootstrap values at major nodes are indicated. Horizontal distances are proportional to the genetic distance between isolates and vertical distances are arbitrary. Scale bar indicates the proportion of sites changing along each branch

Pum: 08: 1 coat protein (AV1) gene, GQ225738] and *Tomato leaf curl Palampur virus* [isolate Varanasi segment DNA-A, FJ931537].

In Neighbor-Joining tree, the *Solanum leaf curl Lakshmangarh virus* (JN009667) placed in a separate monophyletic cluster of 88 bootstrap values and showed genetic relatedness with *Tomato leaf curl Palampur virus* (GQ225738), *Cucurbita pepo begomovirus* (EU366164) and *Tomato leaf curl Palampur virus* (FJ931537) (Fig. 2).

The positive PCR reaction showed the presence of begomovirus, which was further confirmed by Southern blot hybridization using Ageratum enation virus as a general and control probe for begomoviruses. All samples from symptomatic plants hybridized with the probe, whereas samples extracted from non-symptomatic plants did not show positive results.

DISCUSSION

There are reports of begomovirus infection in weed plants worldwide. In regard to Indian context numerous report on begomovirus infection in crops are available[18,19,20,21] but very few knowledge is available regarding begomovirus infections in weed plants[16], this is because weeds are sometimes neglected or are not taken into consideration while carrying out survey and begomovirus studies.

The increasing occurrence of begomoviruses in India demands efforts to study their diversity in order to anticipate and monitor outbreaks as well as to understand the evolutionary forces driving the emergence of novel begomoviruses in ornamental species previously unaffected by these pathogens. This pattern of variation typically occurs due to point mutations in different isolates of a begomoviruses often causing genetic drifts. Comparative nucleotide homology of CP gene sequences indicates that, the genes have different evolutionary origin in the *Solanum nigrum* begomovirus. The possible reason for this difference could be the genetic recombination between begomoviruses[22]. On the basis of the DNA-A sequence comparison and the ICTV demarcation[23, 24] of species at 89% sequence identity; the Indian virus is a provisional strain of *Cucurbita pepo begomovirus* (EU366164) or *Tomato leaf curl Palampur virus* (GQ225738).

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CONCLUSIONS

Many scientific reports have demonstrated that weeds serve as reservoir or alternative hosts for begomovirus survival and spread in the absence of the main crops. Thus, there was a pressing need for additional information on the diversity and distribution of begomoviruses weeds, which likely serve as virus reservoirs. To our best knowledge, this is the first report of begomovirus associated with yellow leaf curl disease of *Solanum nigrum* from Rajasthan, India. These findings suggest phylogeographic evolution of the begomoviruses affecting *Solanaceae* weed *Solanum nigrum*.

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