
Identification of begomovirus-infected mosaic diseases from uncultivated crops of sub-Himalayan plains of East India

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Abstract The common uncultivated plants, *Datura stramonium*, *Jatropha curcas*, *Croton bonplandianum*, *Acalypha indica* and *Ageratum conyzoides* are grown in and around the cultivated fields of sub-Himalayan north-east Indian plains. Chlorosis, leaf curling, distortion, yellowing and stunted growth symptoms are often found in the plants mentioned. Yellow vein mosaic diseases caused by begomoviruses, associated with the above mentioned plants, may act as reservoirs of crop infecting begomoviruses. The core coat protein gene sequences are known to be useful in *Begomovirus* classification and provisional identification of the begomoviruses. To partially characterize the *Begomovirus* complexes associated with the weed species from sub-Himalayan north-east India plains, cloning and sequencing and analysis of the core CP genes were taken into consideration. Three begomoviruses infecting *C. bonplandianum*, *A. indica* and *A. conyzoides* were amplified using two universal primer sets (AV494 and AC1048). Sequence analysis study and phylogenetic analysis of the present *Begomovirus* complexes revealed significant variation among their sequences in spite of their symptom similarities. Interestingly, sequences of the weed infecting viruses exhibited closeness to the sequences of the crop infecting begomoviruses.

Keywords: Uncultivated plants, Begomovirus, core CP.

Introduction

Sub-Himalayan north-east Indian plains comprise the northern part of West Bengal and Brahmaputra valley of Assam. Most of these areas have a climate that is conducive to year-round vegetable production. Substantial losses in farmers' fields have been experienced due to viral diseases during the last several years in the area. High incidence of begomoviruses transmitted by white flies (*Bemisia tabaci*) is one of the formidable biotic constraints of crop

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production in the area. Salati *et al.* (2002) also reported the probable contribution of begomoviruses to the epidemic disease status of cultivated and non-cultivated plants. Other than epidemiological importance, weeds that harbor dual or multiple begomovirus infections may facilitate recombination between the constituent begomoviruses, resulting in the emergence of recombinant viruses (Mendez-Lozano *et al.*, 2002). It is believed that the emergence of tomato-infecting begomoviruses was the result of horizontal transfer of indigenous viruses that infect wild or weed hosts by the new biotype of the whitefly vector. Following entry into new host the indigenous viruses would have rapidly evolved via recombination and pseudorecombination, giving rise to the species currently detected in the field (Castillo-Urquiza *et al.*, 2008). It has also been reported that a number of common weeds serve as alternate hosts as well as reservoirs for many crop-infecting begomoviruses (Roye *et al.*, 1997; Sanz *et al.*, 2000). Several weeds frequently harbor multiple viruses, resulting to the possibility of emergence of new recombinant strains (Mubin *et al.*, 2010; Umaharan *et al.*, 1998). The agro-climatic conditions of north-east India has ideal conditions for plant viruses to attack. Although it has been established that weeds can play an important role in the emergence of plant viral epidemics affecting crops in different parts of the world (McLaughlin *et al.*, 2008; Rojas *et al.*, 2000) but they are still largely neglected.

Only limited work has been carried out to characterize the begomovirus complexes associated with different weed species in India (Paul *et al.*, 2012). As there are reports of weeds, acting as reservoirs facilitating recombination and generation of new viral genomes (Frischmuth *et al.*, 1997, Jovel *et al.*, 2007, Morales and Anderson, 2001) the present study has been taken into consideration. Hence, uncultivated plants like *Datura stramonium*, *Jatropha curcas*, *Croton bonplandianum*, *Acalypha indica*, *A. geratum conyzoides* were considered for detection of viruses. Molecular characterization of the viruses was also thought to be performed using universal begomovirus primers for partial DNA-A genomes.

Materials and methods

Plant Materials

Datura stramonium and *Jatropha curcas* plants showing chlorosis, leaf curling, distortion, yellowing and stunted growth and *Croton bonplandianum*, *Acalypha indica* and *Ageratum conyzoides* (Fig. 1.) showing typical begomovirus infecting symptoms of yellowing of the vein and mosaic were collected from the sub-Himalayan plains of East-India. Altogether 50 samples of the five plants mentioned above were collected. Some infected plants were

selected on the basis of severity of the symptoms and were uprooted from the fields with some soil attached to their roots and immediately placed in plastic pots containing garden soil. All such pots were covered with mosquito net (specially prepared to cover each pot). All the pots thus procured were labeled and maintained in a net house in the experimental garden of the Department of Botany, University of North Bengal.

Primers used

Three set of universal begomovirus primers were used to amplify the partial DNA-A genome of begomoviruses. For preliminary identification of begomovirus infection DengA and DengB (Rojas *et al.*, 1993) universal begomovirus primer was used which amplify the ~530bp region of the DNA-A origin of replication and continues to the 5' end of the AV1 (coat protein) gene. Then a second set of two pair of primers namely CRV301 and CRC1152 which amplify the coat protein gene of all mono- and bi-partite Indian TLCB DNA-A sequences (available in GenBank) and AV494 and AC1048 were also used in the present study which amplify core coat protein gene of all mono- and bi-partite *Begomovirus* DNA-A sequences available in GenBank (Wyatt and Brown, 1996).

DNA sequencing and phylogenetic analysis:

The amplified products of expected size were either directly sent for sequencing or were purified using gel extraction kit (Genei, Bangalore) and cloned into the pGEM T-easy vector (Promega, Madison, USA) following the method of Knoche and Kephart (1999). Sequencing of the clones was done in both direction using universal SP6 forward and T7 reverse primers at Eurofins Genomics India Pvt Ltd. The partial coat protein, coat protein and core coat protein sequence of the infected plants were compared with that of reference begomoviruses obtained from the GenBank data base. BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used for identification of sequence in terms of closest homology and highest nucleotide sequence identity (nsi). CLUSTAL W was used for multiple sequence alignments with the respective begomovirus sequences. MEGA *version* 4.0 software (Tamura and Kumar, 2007) was used for the phylogenetic analysis of core coat protein gene. Phylogenetic tree was constructed by using neighbor-joining method.

Results and discussion

PCR detection of the virus

Three sets of universal degenerate primers were used for amplification and confirmation of the partial coat protein, coat protein and core coat protein genes of viruses from the infected *J. curcas*, *D. stramonium*, *C. bonplandianum*, *A. indica* and *A. conyzoides* plants. Expected amplicon of ~530 bp was successfully amplified using DengA and DengB primers from all the samples to confirm the presence of begomovirus as suggested by several authors. For provisional identification of *Begomovirus* from the infected samples CRV301 and CRC1152 primer pair was used for amplification of coat protein and core coat protein gene. An expected viral amplicon of ~800bp was successfully amplified only from infected plant species *D. stramonium*. On the other hand, an expected viral amplicon of ~575 bp was successfully amplified from infected *C. bonplandianum*, *A. indica* and *A. conyzoides* showing yellowing of the vein and mosaic symptoms.

Analysis of partial coat protein, coat protein and core coat protein gene sequence

In order to know the relationship of begomoviruses infecting the plants in north-east Indian plains the amplified products were cloned and sequenced. After proper annotation and multiple sequence alignment, the sequences were submitted in the GenBank and subsequently received the respective accession numbers. The viral gene isolate from *J. curcas* contained a nucleotide sequence of 495bp (Accession no. HQ597029) containing two ORFs (AV2 and AV1 partial). It showed highest 95% nucleotide sequence identity with *Jatropha mosaic India virus*-[Lucknow] strain SK-2 segment DNA-A (Accession no. HM230683). Bird (1957) and Roye *et al.* (2006) reported the occurrence of *Jatropha mosaic virus* from *Jatropha gossypifolia*, a weed plant from Puerto Rico and Jamaica respectively. The occurrence of begomovirus infection in *Jatropha curcas* causing mosaic and stunting of the plant has been reported by several workers (Raj *et al.*, 2008; Tewari *et al.*, 2007; Gao, 2010). However, the *Jatropha mosaic India virus* (JMIV) infecting *J. curcas* plants and exhibiting mosaic, leaf distortions, curling and blistering have been reported by Narayana *et al.* (2006) from northern India. Similar symptoms have also been observed in *J. curcas* growing in sub-Himalayan West Bengal. *Jatropha* is a perennial weed plant genus and serve as continuous source of inoculum for the virus. Virus from *D. stramonium* having a nucleotide sequence of 771bp (Accession no. JN676054) contained a single ORF of full coat protein (AV1) gene. It showed

highest 99% nucleotide sequence identity with *Tomato leaf curl New Delhi virus* DNA-A complete genome (Accession no. AM850115). Presence of begomovirus infection in *Datura* plant species was reported from India and other parts of the world (Costa, 1955; Marwal *et al.*, 2012; Fiallo-Olive *et al.*, 2013; Ding *et al.*, 2007). Three infected plant (*C. bonplandianum*, *A. indica* and *A. conyzoides*) samples gave positive response of amplification of virus genes (when primers AV494 and AC1048 were used). The GenBank accession numbers of the amplified products were JQ811770 (for 516 nucleotide amplicon from *Acalypha*), JQ796374 (for 555 nucleotide amplicon from *Croton*) and JQ843097 (for 522 nucleotide amplicon from *Ageratum*). The core cp gene of *Acalypha* yellow vein disease-associated complex showed highest (98%) nucleotide sequence identity (nsi) with *Jatropha mosaic India virus* isolate Katarniaghat segment DNA-A (JN135236) and clustered with the same in the phylogenetic tree.

The core CP gene of the begomovirus associated with *Croton* yellow vein disease in the present study area (JQ796374) showed its close relationship (97% nsi) with *Croton yellow vein mosaic virus* (FN645898) which infects *Acalypha* plants in India (Zaffalon *et al.*, 2012) and *Croton* plants in India (JN817516). But in phylogenetic analysis it clustered with begomoviruses infecting papaya, tobacco, mesta and kenaf. The core CP gene of the begomovirus associated with *Ageratum* yellow vein disease of the present study area (JQ843097) showed its close relationship (99% nsi) with both *Tobacco curly shoot virus* infecting *Ageratum* plants in China (AJ971266, FN401522) and with *Tobacco curly shoot virus* of infected pepper (GU001879) plants (Qing *et al.*, 2010). The same core CP gene showed 98% nsi with *Ageratum enation virus* (JF728866) infecting *Ageratum* plants in India and clustering with both the begomoviruses in Fig. 2. A list of viruses along with their hosts and accession numbers used in phylogenetic analysis have been presented in Table (1).

Table 1. Hosts with viruses (accession numbers) used in phylogenetic analysis

Accession No.	Virus Name	Host	Country
AJ971266	<i>Tobacco curly shoot virus</i>	<i>Ageratum conyzoides</i>	China
GU001879	<i>Tobacco curly shoot virus</i>	Pepper	China
FN401522	<i>Tobacco curly shoot virus</i>	<i>Ageratum conyzoides</i>	China
JF728866	<i>Ageratum enation virus</i>	<i>Ageratum conyzoides</i>	India
AM701770	<i>Ageratum enation virus</i>	<i>Brassica rapa</i> subsp <i>rapa</i>	Pakistan

AJ875159	Tomato leaf curl Joydebpur virus	Tomato	Bangladesh
AF314531	Pepper leaf curl Bangladesh virus	Chilli	Bangladesh
EU184018	<i>Acalypha</i> yellow mosaic virus	<i>Acalypha Indica</i>	India
JN807769	<i>Acalypha</i> yellow mosaic virus	<i>Acalypha Indica</i>	India
EU439256	Indian cassava mosaic virus [Lucknow]	<i>Acalypha Indica</i>	India
EU184015	<i>Clerodendron</i> yellow mosaic virus	<i>Clerodendron</i> sp.	India
HQ840737	<i>Jatropha</i> mosaic India virus [Lucknow]	<i>Jatropha curcas</i>	India
JN135236	<i>Jatropha</i> mosaic India virus	<i>Jatropha curcas</i>	India
DQ339122	Whitefly transmitted Indian begomovirus	<i>Phyllanthus niruri</i>	India
FN645898	Croton yellow vein mosaic virus	<i>Acalypha</i> sp.	India
AY007616	Tobacco leaf curl virus	Tobacco	India
JN817516	Croton yellow vein mosaic virus	<i>Croton bonplandianus</i>	India
EU126823	Papaya leaf curl virus	<i>Carica papaya</i>	India
DQ298138	Mesta yellow vein mosaic virus	<i>Hibiscus subdariffa</i>	India
EF620562	Kenaf leaf curl virus	<i>Hibiscus cannabinus</i>	India

Although, weeds are reservoirs of begomoviruses that infect crop plants and act as “melting pots” that yield new viruses/ virus strains by recombination and component exchange due to their frequently harboring multiple viruses, it has been neglected in the study of plant viruses (Mubin *et al.*, 2010). Besides cultivated plants, many weed species are also hosts for begomoviruses (Assuncao *et al.*, 2006) and characterization of those weed-infecting begomoviruses is, therefore important for elucidating their ecology and evolutionary behavior. In the present study, the genomic pattern of begomoviruses of different uncultivated crops has been done with the universal begomovirus specific primers. Several workers used DengA and DengB universal primers to prove the presence of whitefly transmitted begomoviruses (Narayana *et al.*, 2006; Raj *et al.*, 2008; Reddy *et al.*, 2005). As coat protein genes are highly conserved among begomoviruses, the identity of the begomoviruses can be predicted through CP gene sequence analysis when

complete DNA-A sequence is unavailable. Reddy *et al.* (2005) used CRv301 and CRv1152 to detect the diversity and distribution of tomato infecting begomoviruses in India. The core CP primers have been illustrated to amplify a fragment for most, if not all, begomoviruses irrespective of Old or New World origin, making possible the rapid detection followed by prediction of provisional species affiliation by comparing with reference begomovirus core CP sequences (Wyatt and Brown, 1996; Brown *et al.*, 2001). All begomoviruses code for coat protein which act as protective coat of the virus particle and determine vector transmissibility of the viruses by whitefly vector *B. tabaci*. Thus, the CP gene is highly conserved among begomoviruses originating from the same geographical region and adapted to transmission by local vector population (McGrath and Harrison, 1995; Maruthi *et al.*, 2002).

Smaller fragments comprising the core coat protein gene (core CP) sequence have also been used to establish provisional species identification owing to the highly conserved nature of the viral CP sequence (Govindappa *et al.*, 2011). Besides coat protein and core coat protein genes, the 200 nucleotides at the 5' region of the CP gene is highly variable and has been proposed as an informative region for prediction of taxonomic relationships of *Begomoviruses* (Brown *et al.*, 2001; Padidam *et al.*, 1995), although definite species assignment requires sequencing of complete DNA-A, especially due to the high recombination rate of begomovirus genome (Preiss and Jeske, 2003). The presence of recombinant event in the isolated viruses of the present study cannot be nullified. However, considering the high degree of identity among most isolates and previously characterized begomoviruses, tentative species assignments are possible quite reliably as suggested by Rodriguez-Pardina *et al.* (2006). In the present study area *Jatropha*, *Datura*, *Croton*, *Ageratum* and *Acalypha* plants are naturally grown as uncultivated crops and these plants have been taken into consideration for analysis of begomovirus complexes. From the present study, it is evident that *Jatropha* mosaic begomovirus complexes, Tomato leaf curl begomovirus complexes (specially Tomato leaf curl New Delhi virus), Croton yellow vein mosaic begomovirus complexes and Tobacco curly shoot begomovirus complexes (specially Tobacco curly shoot virus) are present in the study area. The associated begomovirus complexes not only infected uncultivated crops but there are a number of reports of association of these viruses with crop infection. *Tomato leaf curl New Delhi virus* is a major pathogen of tomato in India (Padidam *et al.*, 1995; Srivastava *et al.*, 1995). *Tomato leaf curl New Delhi virus* not only infect tomato but also other cultivated crops (Jyothsna *et al.*, 2013). Similarly, *Tobacco curly shoot virus* was also isolated from crops like tomato, pepper, common bean etc. (Li *et al.*, 2005; Qing *et al.*, 2010, 2005; Venkataravanappa *et al.*, 2012). Interestingly,

Croton yellow vein mosaic begomovirus complexes, a major pathogen of *Croton*, other uncultivated weeds (Snehi *et al.*, 2011; Zaffalon *et al.*, 2012; Paul *et al.*, 2012) and of tomato (Pramesh *et al.*, 2013) was also present in the present study area. This study support the fact that uncultivated crops grown around the crop growing fields and associated weeds serve as a reservoir of multiple crop infecting begomoviruses. It was also evident from the study that similar symptoms were not produced by same viruses in different plants. Based on the above analysis, it may be suggested that the existence of genetic diversity of uncultivated crop infecting begomoviruses in sub-Himalayan north-east Indian plains need further investigation. Such knowledge will aid the development of control strategies for virus protection of cultivated crops.



Fig. 1. *Croton bonplandianum* (A = healthy; B = infected), *Acalypha indica* (C = healthy; D = infected), *Ageratum conyzoides* (E = healthy; F = infected), *Datura stramonium* (G = healthy; H = infected) and *Jatropha curcas* (I = healthy; J = infected).

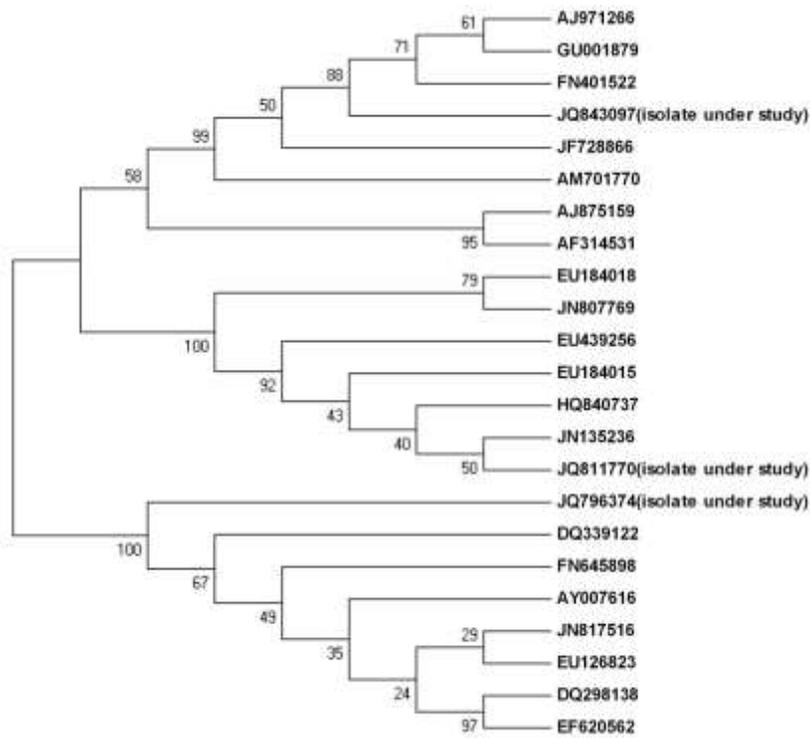


Fig. 2. Most parsimonious tree showing the relationship of core coat protein gene of the isolates collected from sub-Himalayan plains of East India with other GenBank-published begomovirus sequences. Numbers at nodes indicate the bootstrap percentage scores out of 1000 replicates.

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