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Short Communication

Chili Leaf Curl Betasatellite Enhances Symptoms Induced by Tomato Leaf Curl New Delhi Virus, a Bipartite Begomovirus

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Abstract

Begomovirus disease complexes are the major limiting factor on chilies throughout the Indian subcontinent. The severe symptoms in chilies are associated with multiple begomovirus components. Infectivity assays of Tomato leaf curl New Delhi virus (ToLCNDV) and Chili leaf curl betasatellite (ChLCB) were conducted. DNA A and DNA B of ToLCNDV isolated from chilies and tomato were infectious and produced leaf curl symptoms when inoculated on *Nicotiana benthamiana* by biolistic gun method. Co-inoculation of ToLCNDV with ChLCB resulted in the severity of disease symptoms. These results show that interaction of betasatellite with bipartite begomoviruses may enhance symptoms induced by bipartite viruses. Thus interactions of betasatellites and symptom enhancement are not limited to monopartite begomoviruses and bipartite begomovirus-betasatellite interaction presents yet another example of rapid changes in begomovirus complexes that infect important crops in the region. © 2014 Friends Science Publishers

Keywords: Begomoviruses; Chilli pepper; Betasatellite; Disease complex

Introduction

Geminiviruses are circular, single-stranded (ss) DNA plant viruses and have emerged as important pests globally due to their devastating effects on food and fiber crops (Hanley-Bowdoin *et al.,* 1999; Nawaz-Ul-Rehman, 2009). Genus Begomovirus comprises the largest group of plant viruses in family *Geminiviridae*. Several factors such as the availability of large population of insect vector, wide host range, emergence of new viral strains/species due to recombination and dissemination of begomoviruses due to human activity have contributed to their spread and losses caused by these viruses (Varma and Malathi, 2003). Bipartite begomoviruses consist of two genomic components called DNA A and DNA B. DNA A encode proteins that are responsible for encapsidation of viral genome, controlling of gene expression (Sanderfoot and Lazarowitz, 1995), the insect transmission and DNA replication (Laufs *et al.,* 1995). DNA B is responsible for the symptom expression and systemic movement of the virus (von Arnim and Stanley, 1992). Monopartite viruses consist of a single genomic component equivalent to DNA A that carries all the genes responsible for the successful establishment of the virus in the host (Stanley *et al.,* 2005). The vast majority of monopartite begomoviruses are associated with a DNA satellite called betasatellite and these begomovirus-betasatellite complexes constitute the largest group of begomoviruses (Mansoor *et al.,* 2003). Betasatellites are sequence unrelated molecules of approximately half of the size of begomovirus (~1.4 kb) which require helper begomoviruses for replication and encapsidation. In turn these satellites assist their helper viruses in movement and symptom induction by suppressing the RNA-based host defense response (Zhou *et al.,* 2003).

Chilies commonly known as hot peppers were first introduced into Indian subcontinent from Brazil in the 16th century by the Portuguese (Tripathi *et al.,* 2006). Chilli pepper is an important vegetable crop in the Indo-Pak region that often shows symptoms similar to tomato leaf curl, such as yellowing, severe leaf curling, reduction in leaf size, distortion and stunting. Both bipartite and monopartite begomoviruses are associated with the disease in the Indian subcontinent (Hussain *et al.,* 2004; Chattopadhyay *et al.,* 2007). Chilies with severe symptoms are associated with a bipartite begomovirus and a betasatellite (Akhter *et al.,* 2009). The objective of these studies was to perform infectivity assays to show that symptoms induced by ToLCNDV are enhanced by Chilli leaf curl betasatellite (ChLCB).

Materials and Methods

The cloned DNA components reported previously (ToLCNDV DNA A, ToLCNDV DNA B and ChLCB) were restricted from their vectors by using restriction endonuclease enzymes which were incorporated into primers (Akhter *et al.,* 2009). The restriction enzymes used for DNA A and DNA B were MluI and XbaI, respectively, and KpnI was used for ChLCB, which yielded full length

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viral genomic DNAs. *Nicotiana benthamiana* plants were biolistically inoculated with cloned component (Rothenstein *et al.,* 2005). DNA coated gold particles were delivered into *N. benthamiana* plants using gene gun (BioRad).

Genomic DNA of symptomatic plants was extracted from systemic leaves by CTAB method (Doyle and Doyle, 1990). Virus was detected in the genomic DNA of systemic leaves by PCR using primers specific for each component (Akhter *et al.,* 2009).

Results

Plants inoculated with ToLCNDV components from tomato and chilies cloned through PCR and Phi produced the symptoms 18 days post-inoculation (dpi) (Table 1). Plants inoculated using tomato derived ToLCNDV DNA A, DNA B and ChLCB from chilies produced leaf curling and vein thickening symptoms. These symptoms started appearing 15 dpi and their severity kept on increasing with each passing day. While the biolistic inoculation of plants with all the chilies derived viral components (ToLCNDV DNA A, DNA B and ChLCB) resulted in severe leaf curling symptoms and yellow mosaic, which were very close to the symptomatic chilies plants observed in the field (Fig. 1). To check the systemic movement of betasatellite in biolistically inoculated *N. benthamiana* plants, systemic leaves were detached and their genomic DNA was extracted. This extracted DNA was then subjected to PCR using same set of primers (Akhter *et al.,* 2009) which were previously used to clone the ChLCB. PCR products on agarose gel showed a single band of approximately 1400 bp of size (Fig. 2). This result confirmed that betasatellite is moving in the systemic leaves along with DNA B and also increasing the symptom severity.

Discussion

Maximum molecular diversity of geminiviruses and their associated satellite components exist in Southeast Asia (Nawaz-Ul-Rehman, 2009; Akhtar *et al.,* 2013). Begomoviruses acquired betasatellite components at some point during their evolution, although its origin remains unknown (Mansoor *et al.,* 2003). Betasatellites are pathogenicity determinants associated with monopartite begomoviruses in the old world (Briddon and Stanley, 2006). Betasatellites are totally reliant on the helper component (DNA-A) for their replication, en-capsidation and transmission by whitefly vectors (Briddon *et al.,* 2003). Association of betasatellites with their cognate helper viruses is not very strict. Several examples can be found for the relaxed adaptation of betasatellites with helper begomoviruses. Recently Cotton leaf curl Gezira virus (CLCuGV) has been found in southern areas of Pakistan without its cognate betasatellite. Local betasatellites of Asian origin CLCuMB (Cotton leaf curl Multan betasatellite) and ChLCB were found associated with CLCuGV (Tahir *et al.,* 2011). Earlier it has been noted that heterologous betasatellite (CLCuMB) can substitute the movement function of DNA B of bipartite begomovirus (Saeed *et al.,* 2007). In another study, ToCNDV DNA A and CLCuMB were co-inoculated in cotton and mild leaf curl symptoms were induced, which indicated that CLCuMB can be transreplicated by ToLCNDV DNA A (Saeed, 2010). The bipartite Pepper leaf curl virus has residual ability to interact with betasatellites and mild symptoms on infected plants were witnessed as demonstrated by Shafiq *et al.* (2010). Here we report for the first time that the association of betasatellite (ChLCB) with ToLCNDV in a synergistic manner, which increased the symptom severity to a greater extent (Fig. 1). However the DNA B and betasatellite are probably evolutionary unrelated and simply represent functionally convergent disease complex. Though this bipartite begomovirus has not been identified in cotton yet, its successful combination with cotton isolated betas promises a serious threat.

Taken together, these results demonstrate that the epidemics caused by begomoviruses in chili crop is associated with a high level of complexity caused by the begomovirus species involved and the sequence diversity of the begomovirus species. Available evidence suggests that these disease complexes are rapidly expanding in terms of their geographical distribution and host range (Mansoor *et al.,* 2003). As bipartite begomoviruses are more efficient in increasing their host range (Akhtar *et al.,* 2011), the association of betasatellite may help them increase this efficiency.

Future studies should address the epidemiological consequences of the observed virus diversity. Appearance of chili leaf curl disease in such a great severity can be attributed to the synergistic action of geminivirus disease

Table 1: Summary of infectivity studies

Treatments	treatment used for bombardment	Total plants bombarded	Symptomatic plants	PCR detection of ChLCB	Symptoms/panels in Fig. 1
1	ToLCNDV DNA A + DNA B (both components from tomato)	10	10		Leaf curling and vein thickening /A
2	ToLCNDV DNA A + DNA B (both components from tomato) + ChLCB (chilies)	10	10	+	Severe leaf curling and thickening/B
3	ToLCNDV DNA A + DNA B + ChLCB (all the components from chilies)	10	10	+	Sever leaf curling, vein thickening and mosaic pattern/C
4	Non-inoculated control	05	00		No symptom/D

+amplification of betasatellite (1.4 kb)

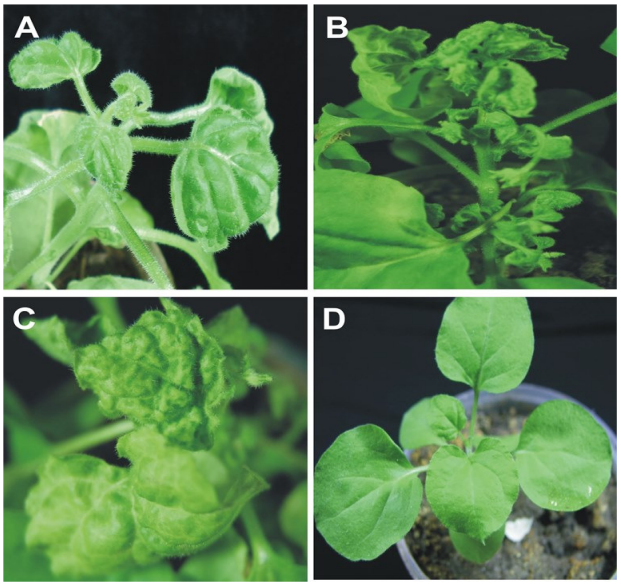

Fig. 1: Photographs of *N. benthamiana* plants biolistically inoculated with ToLCNDV DNA A and DNA B and both components were cloned from tomato (panel A), ToLCNDV DNA A, DNA B from tomato and ChLCB from chilies (panel B), ToLCNDV DNA A, DNA B cloned from chilies and ChLCB (panel C) and non-inoculated healthy plant of *N. benthamiana* (panel D). Photographs of *N. benthamiana* plants were taken 18 dpi

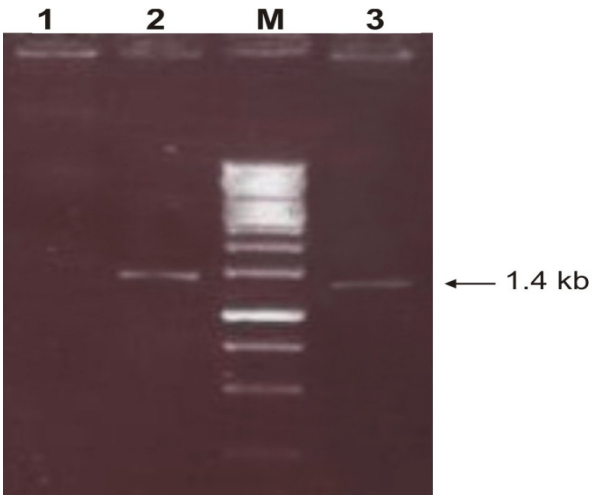

Fig. 2: PCR mediated detection of ChLCB in inoculated *Nicotiana benthamiana* plants. Ethidium bromide-stained agarose gel was illuminated under UV light and photograph was taken. Well 1 contains PCR product resulting from template DNA from non-inoculated negative control plant. Genomic DNA of plant inoculated with ToLCNDV DNA A, DNA B (from tomato) and ChLCB (from chilies) was loaded in well 2 and ToLCNDV DNA A, DNA B and ChLCB (from chilies) in well 3. Well M represents 1 kb DNA ladder. Genomic DNA was extracted from plants 18 dpi

complex comprising a bipartite virus along with a betasatellite component.

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