Research paper

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Impact of Pepper Leaf Curl Virus and *Candidatus* Phytoplasma Asteris in Plants

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ABSTRACT

This research has reviewed about pepper leaf curl virus and candidatus phytoplasma and its impacts in plants. A number of related work has been done by various researcher across the world. Phytoplasmas are bacteria that infect plants and require both a host plant and an insect vector to propagate and survive. Similarly, pepper leaf curl virus is a significant constraint on plants induced by begomovirus. Begomoviruses are belonging to the geminiviruses family that are mostly transmitted by whiteflies from one plant to other. Their genomes contain two components (DNA A and B) or one (DNA C) (homologous to the DNA A and B component of bipartite begomoviruses). These viruses changes physiological responses in plants via signaling of hormonal, nutritional and stress where as induce their interactions in the environment.

KEYWORDS

Pepper Leaf Curl Virus, Phytoplasma, Plants, Geminiviridae, pathogens, bacterial plants.

INTRODUCTION

Vegetables are short-duration crops that are grown throughout the year and yield a high economic return. PepLCV is a DNA virus that belongs to the genus Begomovirus (Plant Viruses). It is also a member of the family Geminiviridae (encode their genetic information on a circular genome). Due to the distinct molecular evolutionary histories of the two genetic components, they are expected to undergo divergent evolutionary pressures. DNA B began as a satellite that was captured and developed into a key genome component by the monopartite progenitor of all currently known bipartite begomoviruses.

Vegetables are impacted by a variety of abiotic and biotic variables, and phytoplasmaassociated diseases are a substantial constraint in a number of regions of the world, resulting in significant production and quality losses. Phytoplasmas are prokaryotic bacteria that thrive and proliferate in the isotonic environments of plant phloem and insect haemolymph.



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Phytoplasmas have been associated to over 600 plant diseases. Leafhoppers and plant hoppers are the most prevalent phloem-feeding insects that disseminate these infections ("Bertaccini et al., 2014").

Bitter gourd ("Momordicacharantia L."), sometimes known as bitter melon, is a Cucurbitaceae family member that is widely cultivated in China, India, and Southeast Asia. Furthermore, it is grown on a small scale in California and Florida, United States of America, for culinary and medicinal purposes. In traditional Indian, Chinese, African, and Latin American medicine, bitter gourd has a long and illustrious history of use. There is evidence that its extract contains anti-oxidant, antibacterial, antiviral, anti-hepatotoxic, and antiulcerogenic qualities, among other things ("Behera et al. 2009"). Bitter gourd is a popular vegetable crop in Uttar Pradesh (UP), India's northeastern region, and is a significant commercial crop ("Yadav et al. 2004").

This research has focused on assessing the impacts of pepper leaf curl virus and candidates phytoplasmasteris on plants. Hence this research has focused on the reviewing the researches with that it can be found out how pepper leaf curl virus and candidatusphytoplasmaasteris affects the plant.

Causes and Symptoms of Pepper Leaf Curl Virus

Causes of Pepper Leaf Curl Virus

Peppers add heat and a huge range of colors to the vegetable garden, but like their cousins the tomatoes, they can be finicky about growing conditions and sensitive to pest damage. Curled leaves of peppers, as well as tomato plants, are a common sign. Hence, there are three major issues that causes pepper leaf curling (Kristi Waterworth, 2013):

- Viral diseases can cause curling leaves on peppers, among other symptoms like yellow spots, rings, or bullseyes on leaves as well as general un-thriftiness. Insect pests carry viral agents between plants, spreading these incurable diseases far and wide. If you suspect a virus, immediately remove the infected plant to help prevent further disease spread and keep pests under control. Viruses aren't usually present in the soil, so if you catch it early in the season, you may be able to replace the affected plants. Virus-resistant peppers are available from most nurseries for gardens with recurrent virus problems.
- Environmental problems are often at the root of pepper plants with leaf curl. Pepper leaf curl regularly appears on hot days, during the middle of summer; hot winds combined with low humidity cause leaves to cup in self-defense. If leaves curl only in response to heat, try adding extra water during the middle of the day to keep the plant's tissues cooler.



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• By feeding on pepper plants, pests such as aphids, thrips, mites, and whiteflies induce leaf curl. Leaves that are fed during development curl or twist at random, depending on the location, and can become speckled or stippled as they mature. As a result of sap-feeding, many of these pests create honeydew, a sticky, sweet substance—a shiny transparent coating of material can be seen near feeding locations.

Overviewof Symptoms of Pepper Leaf Curl

Three hundred and seven genotypes were tested for resistance to the virus that causes chilli leaf curl disease, pepper leaf curl virus (PepLCV) ("Capsicum annuum"). The initial screening was carried out in the field, and illness reactions were assigned to each genotype based on the coefficient of infection (CI). As a result, in a glasshouse setting, selfed progenies of eight symptom-free and highly resistant lines were challenged with viruliferous white fly, with just three genotypes exhibiting no symptoms: GKC-29, BS-35, and EC-497636 demonstrating no symptoms. Following grafting and alternating grafting with the scion and root stalk of a sensitive genotype, these three apparently symptom-free genotypes were examined. They were grafted and alternate grafted with the scion and root stalk of a sensitive genotype (PusaJwala). Effective/alternative grafting was used for 50 days to verify that there were no viral symptoms on grafted plants from any of the genotypes tested. The absence of viral symptoms on plants from any of the genotypes tested confirmed the resistance reactions of GKC-29, BS-35, and E-497636. Amplification of the three symptom-free genotypes was not possible using degenerate primers designed to detect gemniviruses such as PepLCV, indicating that the resistant reaction in the three identified symptom-free resistant sources was caused by a lack of viral genome and that they are not symptom-free carriers of PepLCV, as previously suggested ("Kumar S et al. 2006").



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Figure 1. Symptoms of Pepper Leaf

Infectivitywith Symptoms of Pepper Leaf Curl Virus

241 nucleotides long, it matches the ToLCNDVintergenic regions (IRs) mentioned in Table 1. Agrobacterium-mediated inoculation was used to test PepLCLV clone PGL1's infectivity in "*Nicotianabenthamiana*, *Nicotianatabacum Samsun*, *and C. annum*" (Table 2).

Table 1. The nucleotide sequences of the genomic components and the amino acid sequences of Begomovirus are identical

Begomovirus	"Complete Sequence (percentage nucleotide sequence identity) "	"Intergenic Region (percentage nucleotide sequence identity"	"Gene# (percentage amino acid sequence identity)"					
			AV2	СР	REn	TrAP	Rep	AC4
PapLCV [5]*	72.3-87.0	61.9-85.9	69.1- 90.5	92.5- 97.6	73.5- 90.9	70.5- 95.5	61.3- 91.0	40.5- 94.0
PepLCV [4]*	74.0-86.7	51.7-80.4	75.0- 91.4	78.1- 98.0	66.7- 90.9	71.2- 91.7	69.6- 75.8	34.5- 35.7

^{*} Numbers of sequences from the databases used in the comparisons.

Table 2. Infectivity and Symptoms of Pepper Leaf Curl Virus

Plant Species	Inoculum		Infectiv	Symptoms			
		I	II	III	IV	Total	
N. benthamiana	"PepLCLV"	2/10	1/6	0/7	1/5	4/28	very mild leaf curling
	"PepLCLV + ChLCB"	1/10	0/6	1/7	0/5	2/28	very mild leaf curling
	"PepLCLV + ToLCNDV DNA B"	9/10	6/6	6/7	4/5	25/28	severe downward leaf curling
	"PepLCLV + ToLCNDV DNAB + ChLCB"	8/10	5/6	7/7	4/5	24/28	severe downward leaf curling
	"PepLCLV + CLCuMB"	0/10	-	-	-	0/10	no symptoms
N. tabacum	"PepLCLV + ChLCB"	0/10	0/4	-	-	0/14	no symptoms
	"PepLCLV + ToLCNDV DNA B"	8/10	3/6	-	-	11/16	leaf curling
C. annum	"PepLCLV + ChLCB"	0/10	0/5	-	-	0/15	no symptoms
	"PepLCLV + ToLCNDV DNA B"	5/10	3/6	-	-	8/16	leaf curling

^{*}Shafiq et al. Virology Journal 2010

Reports' Review on Pepper Leaf Curl Virus

There are many different kinds of pepper leaf curl viruses have been found in the plants those have been explained below:



[#]Gene names are as indicated

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On Winter Cherry, the Pepper Leaf Curl Lahore Virus

Using Begomovirus-specific primers, researchers detected 1.2 kb amplicons in naturally and experimentally infected "S. capsicastrum" plants ("Rojas et al., 1993"). The 2740 bp DNA-A molecule was amplified, cloned, and sequenced to identify the Begomovirus (Accession No. "PepLCLV (AM691745), JN880419). It was compared to PepLCLV-[Pakistan:Lahore1:2004] (AM404179). PepLCLV-[Pakistan:Lahore2:2004] and (AM491589)", all from Capsicum annuum. "HM007101, HM007096, and DQ116881" matched isolates of Pepper leaf curl Bangladesh virus from "C. annuum" in India and a ratio 90-91. "Fauguet et al. (2008)" Pakistan of capsicastrumBegomovirusPepLCLV-[Solanumcapsicastrum: Lucknow, India]. PepLCLV- [S. capsicastrum]" variant clustering within a single distinct branch that also contains PepLCLV (Figure 2). PepLCLV is a virus found in Pakistan on "C. annuum", while Eggplant mottled crinkle virus is found in India on S. capsicastrum ("Tahir et al., 2010") ("Raj et al., 1988"). However, India is the first country to report an infection of S. capsicastrum with a Begomovirus.



Figure 2.Infected Solanumcapsicastrum with leaf curl disease (a) and green blisters and deformation (b).

On Chilli Plants, the Pepper Leaf Curl Lahore Virus

Tomato leaf curl New Delhi virus, a begomovirus, and the virus' DNA B component were found in a chilli plant (Capsicum annum) (ToLCNDV). The begomovirus genome is 2747 nucleotides long and shares 99 percent of its sequence with the Lahore virus that causes



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pepper leaf curls ("PepLCLV-[PK: Lah:04], acc. no. AM404179"). The clone was administered into Nicotianabenthamiana using Agrobacterium, resulting in little symptoms and detected viral Polymerase chain reaction analysis of DNA extracted from systemically infected leaves (PCR). Nicotianatabacum or chilies with or without a betasatellite exhibited no symptoms or consequences. Inoculating PepLCLV with ToLCNDV's DNA B component affected Nicotianabenthamiana, Nicotianatabacum, and chiles. More viral DNA was gathered by plants with PepLCLV and ToLCNDV's DNA B component than PepLCLV alone.

Chilli leaf curl disease (ChLCD), caused by begomoviruses, and is a serious threat to the Indian subcontinent's chilli economy ("Briddon RW et. al. 2003; Shih SL et al. 2003; Senanayake DMJB et al. 2006"). The disease is characterised by severe leaf curl, cup-shaped, upward curling leaves, yellowing, and restricted plant development. The discovery of the chilli leaf curl betasatellite (ChLCB) in a large collection of chilli samples from Pakistan that had been infected with leaf curl ("Hussain M et al. 2009"). Briddon RW et al. (2003) identified a single betasatellite species (ChLCB) linked with geographically isolated isolates and identical to a previously described species ("Briddon RW et al. 2003"). Yellowing, leaf curving, reduced leaf size, and stunting are common symptoms of chilli pepper leaf curl disease. Chilli peppers may become infected with tomato begomoviruses in regions where chilli and tomato crops coexist. The whitefly Bemisiatabaci was used experimentally to transmit the illness from diseased to healthy chilli and tomato plants ("HussainM et. al. 2004; Khan MS et al. 2006"). On injected chilli plants, typical disease symptoms were observed. On the other hand, tomato plants that had been injected had severe leaf curl symptoms comparable to those associated with tomato leaf curl disease caused by the Tomato leaf curl New Delhi virus (ToLCNDV) ("Khan MS et al. 2006; Akhter A et al. 2009"). A vast collection of Pakistani chilli samples showed begomoviruses can infect chiles ("Shih SL et al. 2003"). Another begomovirus, PepLCLV, was recently discovered in Pakistani pepper, but its ability to infect chilies has yet to be determined ("Tahir et al. 2010").

Genetic Diversity of Pepper Leaf Curl Virus

Chilli leaf curl virus (ChLCV), formerly thought to be restricted to the Indian subcontinent, has been linked to tomato and pepper illnesses across Oman's vegetable-growing regions. Additionally, a beta satellite was detected in some plant samples that were infected. We sequenced a total of 19 begomovirus clones with the potential to be full-length and eight beta satellite clones. The begomovirus clones share a nucleotide sequence identity of better than 96 percent, indicating that they belong to a single species. The virus discovered in Oman has the highest nucleotide sequence identity (88.0-91.1%) with Pakistani isolates (ChLCV-PK), showing it is a unique strain designated as the Oman strain (ChLCV-OM). A recombination study concluded that ChLCV-OM arose through recombination between ChLCV-PK (the



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primary parent), pepper leaf curl Lahore virus, and a third ChLCV strain. The beta satellite sequences discovered were strikingly similar to ToLCB isolates identified in Oman. An Agrobacterium-mediated viral and beta satellite clone inoculation caused sickness in tomato. This indicated that the existence of the beta satellite exacerbated the virus's symptoms, despite the fact that viral DNA levels remained unaltered. ChLCV-OM is Oman's fourth known begomovirus and the first in Capsicum.

Begomoviruses ("genus Begomovirus; family Geminiviridae") have expanded throughout the subtropics and tropics over the last few decades, wreaking havoc on agriculture. ("Brown, 1997; Moffat, 1999; Varma and Malathi, 2003"). They infect dicotyledonous plants and are regularly transmitted by whiteflies belonging to the Bemisiatabaci species complex ("Brown et al., 1994; Jones, 2003"). American begomoviruses contain a bipartite genome of circular single-stranded DNA. The Americas' begomoviruses contain a bipartite genome made up of circular single-stranded DNA ranging in size from 2•6-2•7 kb("Brown, 1997; Varma and Malathi, 2003"). Numerous begomoviruses have arisen throughout Central America, Mexico, the Caribbean Basin, and the southern United States over the last 25 years, wreaking havoc on a variety of crops such as tomatoes, peppers, beans, and cucurbits ("Brown and Bird, 1992; Polston and Anderson, 1997; Morales and Anderson, 2001; Jones, 2003"). Local populations of "B. tabaci" may have moved due to intensive crop monocultures, pesticide tolerance development, and the newly introduced B biotype of B. tabaci having a larger host range ("Torres-Pacheco et al., 1996; Morales and Anderson, 2001").

Begomoviruses were found in Nicaraguan tomato ("Lycopersiconesculentum") fields and pepper ("C. baccatum"), nearby pepper ("Capsicum annuum"), chilli ("Cucurbitaargyrosperma"), and Mexican fireplant ("Euphorbia heterophylla") plants using polymerase chain reaction (PCR). Symptomatic tomato and Mexican fireplant plants tested positive for begomoviruses, although only 30%-46% of pepper, chilli pepper, and cushaw plants did. Potatoes were begomovirus free. The viral coat protein gene (533 bp) was sequenced to identify the virus species (AV1). Tomatoes and peppers were discovered to be infected with ToSLCV, ToLCSinV, and PGMV, respectively (PepGMV).

Begomoviruses devastate Nicaraguan tomato yields, and whiteflies on infected plants can reach epidemic proportions ("Polston and Anderson, 1997; Rojas et al., 1993; Morales and Anderson, 2001"). Whiteflies and symptoms in other crops such as pepper, chilli pepper, cucurbits, and potato suggest begomovirus infestation. In Nicaragua, the same viruses infect different host species may co-infect any of the hosts, however this is unknown. To identify begomovirus infections in diseased plants near tomato fields, collect data on possible mixed viral infections in tomato and other plants, analyse virus sequence variability among affected plants, and contribute to the body of knowledge about begomovirus diversity in Nicaragua.



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Sixty-nine pepper plants (Capsicum annuum) presenting symptoms were sampled in Managua (CNIA), Sebaco, and Tecolostote ("*Universidad NacionalAgraria-UNA*"). All chilli plants had mosaic, interveinal yellowing, twisted leaves, leaf rolling, and severe stunting. A total of 26 plants were collected in Managua and Las Playitas, Nicaragua. The viral symptoms included mosaic and leaf deformation in all cucurbit plants except one in CNIA. E. heterophylla plants were found in Sebaco and Tecolostote. Plants with virus-like symptoms like yellow mosaic, leaf twisting and deformation, and plant stunting were sampled in Matagalpa. The plants were shipped to UNA on ice and kept at 20°C until examined ("*M. Ala-Poikela, E. Svensson, A. Rojas, T. Horko, L. Paulin, J. P. T. Valkonen, and A. Kvarnheden, 2005*").

The study ("Muhammad Shafiq, ShaheenAsad, Yusuf Zafar, Rob W Briddon, and ShahidMansoor, 2010") demonstrated that the PepLCLV isolate investigated here is incapable of trans-replicating betasatellites, or has a restricted ability to do so. This is in contrast to the findings of Tahir et al., who found a link between ChLCB and another PepLCLV isolate from Pakistan. In addition to an N-terminal leader sequence (Figure 2), the virus's putative Rep protein lacks a trans-replication leader motif. Aside from confirmation, this notion needs be confirmed through mutagenesis. The third possibility is that natural PepLCLV variants exist that lack the Rep protein's leader peptide and so can interact with betasatellites. Given their recent lack of thoroughness in characterising begomoviruses, it wouldn't be surprising to find another one among naturally infected chilies. Chilli leaf curl disease (ChLCD) is a major production hindrance in Pakistan and India. Begomovirus genomes can be bipartite (DNA A and B) or monopartite (only DNA A) (collectively known as beta satellites). Chile previously recognised all three categories, formerly known as ("Akhter A et al. 2009"). PGL1 (Punjab begomovirus) was cloned and found to have 2747 nucleotides. The genome shares 99 percent sequence similarity with ("PepLCLV-[*PK:Lah:04*]") ("Fauguet al. 2008"). **Symptoms** of Agrobacterium et Nicotianabenthamiana Soil-injected betasatellites from ChLCD-affected plants showed no viral infection. The ToLCNDV DNA B infection of "N. benthamiana, N. tabacum, and C. annum mimicked ChLCD". These findings show that the virus is bipartite. ToLCNDV DNA B was substantially less prevalent in plants treated with PepLCBDV. (Fig. 3)



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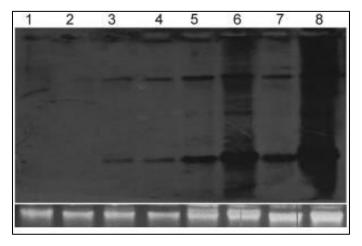


Figure 3.PGL1 examined virus proliferation in inoculated N. benthamiana systemic leaves (**Source:** Experimental Work by Shafiq*et al.* 2010)

This is the first time that an infectious bipartite begomovirus has been shown to generate ChLCD in an experimental setting. As with ToLCNDV DNA B, in Pakistan, a bipartite PepLCLV variant associated with a beta satellite (ChLCB) may result in ChLCD ("Tahir et al. 2010"). Both PepLCLV isolates can connect to ChLCB, possibly due to the presence of Rep N-terminal amino acid sequences in the strain presented here. The method by which begomovirus-encoded Rep interacts with beta satellites to drive satellite replication is unknown ("Saunders et al. 2008"). PepLCLV can trans-replicate ToLCNDV DNA B and generate ChLCD in experimental hosts and chilies. First, Koch used cloned viral DNA components from a bipartite begomovirus that generates ChLCD. Having both a DNA B and a beta satellite appears to lower viral titre and severity in plants. The intricate nature of ChLCD over the Indian subcontinent, which has been connected to a number of bipartite and monopartite beta satellite-associated begomoviruses, would make developing resistant cultivars by conventional or non-traditional ways difficult. Significant productivity losses due to ChLCD are jeopardisingchilli agriculture and pushing farmers in some locations to switch to alternative crops.

PhytoplasmaImpacts on Plant

PhytoplasmaDisease – Review

Phytoplasmas infect plants and insects. They are disseminated by insects, which inject the pathogen into the plants' phloem during their feeding activities. The pathogen manifests a variety of symptoms, the most of which are potentially detrimental to plant health. Phytoplasmaare bacteria that dwell in a plant's phloem cells and frequently, but not always, cause disease symptoms. Phytoplasmas are bacteria that can only be found in the phloem tissues of plants. Because of their inherent parasitic nature, large quantities of them cannot be



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produced in vitro ("Contaldo et al., 2012, 2016"). We only have a fragmented and often indirect understanding of their biology due to known genome sequences ("Shigeyuki and Yasuko, 2015") and/or transcriptome, proteomic, and metabolomic investigations of diseased plants ("Ehya et al., 2013; Abbà et al., 2014; Gai et al., 2014b; 2018b").

Phytoplasma infections were initially categorised as auxonic, suggesting that they slowed the plant's growth ("Horsfall and Dimond, 2012"). Phytoplasmas cause harm via effector proteins that have a significant and diversified effect on not just plant development but also other elements of plant life. Phytoplasma effectors in phloem sieve cells ("Bai et al., 2009; Hoshi et al., 2009; MacLean et al., 2011, 2014; Kitazawa et al., 2017"). These effector proteins govern physiological processes in plants, causing symptoms in cells that exit the phloem. Symptoms of infection include stalk extension (also known as witches' broom), stunting, yellowing, leaf-like tissue growth in place of flowers (phyllody), and greening of the floral organs (i.e., virescence). Inhibition of hormonal, nutritional, developmental, and stress signalling pathways by phytoplasma appears to be the cause of these physiological conditions.

ControllingPhytoplasma Disease

Controlling phytoplasma diseases usually begins with controlling insect vectors. This starts with good weed removal practices and clearing brush that can host insect vectors. Bacteria in one plant can also spread to other plants, so often removal of an infected plant is necessary to contain the contagion ("Bonnie L. Grant, 2016").

Conclusion

From the research reviews, it was found that based on the genetic diversity of the pepper leaf curl virus the leaf shows number of symptoms as shown in table 1 and 2. From the tables it can be concluded that the leaves are either curling upward and downward due to the viral impact. Given the diversity of begomoviruses found in chilies, we hypothesise that PepLCLV was recently recruited into chilies as a result of its association with ToLCNDV DNA B. Notably, the putative rep-binding iterons discovered on PepLCLV (GGGGAC) differ from those found on ToLCNDV by two base positions (GGTGTC). These impact are due to environmental impact, pests' effect or viral attack on the plants and these impacts on plants are very critical. Additionally, the research has reviewed the candidatusphytoplasma and its impact on plant. Given their peculiar lifestyle and difficulty in culture, Phytoplasma and their impact on plants remain unknown. In addition, newly available transcriptome, proteome, and metabolomic data from phytoplasma-infected host plants offer critical new information. As a result, pepper leaf curl virus and phytoplasma have a deleterious impact on plants.



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