Journal of Agroecology and Natural Resource Management
p-ISSN: 2394-0786, e-ISSN: 2394-0794, Volume 4, Issue 1; January-March, 2017, pp. 90-94

http://www.krishisanskriti.org/Publication.html

A Study on the Diversity of Cotton Leaf Curl Virus Infecting Cotton in India

Taruna Gupta[1] and Abhinav Kumar[2]

[1]Centre for Research Studies, Noida International University, GB Nagar, Greater Noida, UP, Sector-17-A, India
[2]School of Sciences, Noida International University, GB Nagar, Greater Noida, UP, Sector-17-A, India
E-mail: [1]tarunagupta67@gmail.com, [2]abhinavkumar@niu.edu.in

Abstract—*Cotton Leaf Curling Disease (CLCuD), caused by cotton leaf curl virus (CLCuV) is a devastating disease causes small vein thickening type symptom on young leaves of plants, upward or downward leaf curling followed by formation of cup shaped leaf. A member of genus Begomovirus (family Germinivirdae), characterized by twin quasi-isometric particles on their genome arrangement. Every year, it causes tremendous loss to the cotton growing areas in India, ranges from 20% to 80%. Here we are making comparative studies considering complete nucleotide sequence to observe the similarity and dissimilarity between the different isolates of cotton leaf curl virus that has infected cotton plant in various region of India. Evolutionary analyses were conducted in MEGA6.*
Keywords*: Cotton Leaf Curling Disease (CLCuD), Cotton Leaf Curl virus (CLCuV) Begomovirus, MEGA6.*

1. INTRODUCTION

Cotton is a natural fibre of vegetable origin, like linen, jute or hemp. Mostly composed of cellulose (a carbohydrate plant substance) and formed by twisted, ribbon-like shaped fibres, cotton is the fruit of a shrubby plant commonly referred to as the "cotton plant". The cotton plant, a variety of plants of the genus *Gossypium*, belongs to the *Malvacae* family, which comprises approximately 1,500 species, also including the baobab tree, the bombax or the mallow. Although the cotton plant is native to tropical countries, cotton production is not limited to the tropics. Indeed, the emergence of new varieties, as well as advances in cultivation techniques led to the expansion of its culture within an area straddling from approximately 47 degrees North latitude (Ukraine) to 32 degrees South (Australia). Although cotton is widely planted in both hemispheres,it remains a sun-loving plant highly vulnerable to freezing temperatures. Cotton is crucially important to several developing countries. Out of the 85 cotton-producing countries in 2005, 80 were developingcountries, 28 of which were indexed by the United Nations among the least developed countries (LDCs).

Insect pests and diseases have always been the major constraint in the cultivation of cotton in India. The severity of this problem can be gauged from the fact that, cotton is the single largest crop accounting for nearly 50% of the total plant protection chemicals used in the country (Gupta. 2001).Cotton is attacked by a number of insect pests and diseases. The major insect pests are aphids, jassids, whiteflies, thrips, leaf roller, red cotton bug, mealy bug, stem weevil, ash weevil, tobacco cutworm, bollworms and gram caterpillar. The important diseases are root rot, anthracnose, leaf blight, leaf curl, bacterial blight, wilt, *Alternaria* leaf spot, *Cercospora* leaf spot, *Helminthosporium* leaf spot and grey mildew. Cotton cultivation is badly affected by plant viruses and among them members of family Geminiviruses are most important. Geminiviruses are characterised by circular single stranded DNA (ssDNA) genomes encapsidated in twinned quasi isometric particles of about 18 x 30 nm. The Geminiviridae family has been divided into seven genera: *Begomovirus, Mastrevirus, Curtovirus, Topocuvirus, Becurtovirus, Turncurtovirus* and *Eragrovirus* (Brown *et al*., 2012).

In the recent years leaf curl disease of cotton (CLCuD) caused by a whitefly (*Bemisia tabaci*) transmitted geminivirus (WTG), cotton leaf curl virus (CLCuV), is posing a major threat to the cotton cultivation in northern India (Varma., 1994, leuman *et al*., 2010) with the yield losses varying from 68% to 79% depending on the cultivars used. Among begomoviruses, the genome organisation is divided into two components, DNA–A and DNA B.DNA–A is essential for replication and encapsidation (Rogers *et al*., 1986; Townsend *et al.,* 1986; Sunter *et al.,* 1987) and DNA B plays a role in systemic movement and symptom production (Etessami *et al.,* 1988; Noueiry *et al.,* 1994).

DNA–A has two ORFs in the virion sense or rightward direction (*AV1*, coat protein; *AV2*, pre coat protein) and five ORFs in the complementry sense or leftward direction (*AC1*, replication initiator protein; *AC2*, transcription activator protein, *AC3*, Replication enhancer protein, *AC4* and A*C5*). DNA B has one ORF each in virion strand or rightward direction (*BV1*-Nucclear shuttle protein) and complementry strand or leftward orientation (*BC1* movement protein).

The worst affected states being Rajasthan, Haryana, and Punjab. Leaf curl disease of cotton was reported first time in

Gossipium peruvianum and *G.ratifolia* in Nigeria during 1912 (Farquhasson, 1912). This disease had epidemics in Nigeria during 1924 followed by Sudan (1927-28) since then there have been reports from African continent (Cauteaux *et al.*, 1968, Fauquet and Thouvenel., 1987). During the last decade it has caused substantial losses to cotton crops in Pakistan. In India Cotton leaf curl disease (CLCuD) has emerged as a major disease of cotton in Northern India. In India, it was reported for the first time in 1989 on *Gossypium hirsutum* cotton at the Indian Agricultural Research Institute, New Delhi and subsequently in the Sriganga Nagar district of Rajasthan in 1993. Thereafter, it has spread throughout Northern India in a short span of 4–5 years (Monga *et al.*, 2004) and has now become a potential threat in the irrigated cotton production belt of the country. The causal organism of this complex disease is CLCuV and associated satellites (alpha satellite DNA and beta satellite DNA) realizing the potential threats of leaf curl disease of cotton,it is feared that this disease might affect cotton growing areas in India. A timely action is therefore required to design a knowledge-based strategy to control this disease. It is therefore necessary to check the diversity of Cotton leaf curl virus from India.During 2015, several cotton growing district of Punjab were badly affected by this disease due to which loss of heavy counts between 70%-100% were observed in different fields that ultimately result to the suicide of many farmers.

The initiation of disease is characterized by small vein thickening type symptoms on young upper leaves of plants, dark green bead like thickening of small vein. The irregular thickenings gradually extend to form a small vein. The disease is further characterized by upward curling of leaves because of the uneven growth of veinal tissues on the abaxial side of the leaves.

The work described in this paper taken together with previous studies on CLCuV establishes phylogenetic relationship between different isolates of CLCuV from India. Reports of genetic variability among naturally occurring DNA viruses, specifically ssDNA viruses are less than those of RNA viruses. The future prospects of these kind of studies are understanding virus evolution, and also development of transgenic plant through different approaches.

2. METHODS

The complete nucleotide sequences of 21 isolates were initially taken into account to see the similarity and dissimilarity. The sequences were downloaded from Pubmed (https://www.ncbi.nlm.nih.gov/pubmed). Complete nucleotide sequences of all the isolates were aligned using CLUSTALW (Thomson *et al.*, 1994) program. After multiple sequence alignments, phylogenetic analysis was done by using MEGA software version 6.0 (AddRef). Default parameter used was Neighbour-Joining. A consensus dendrogram was generated using bootstrap value of 1000 replicates for these algorithms. GenBank accession numbers of different begomovirus strains used for this study are given in Table 1. A separate study was also done for AC1 (replicase) and AV1 (coat protein) regions.

3. RESULTS

Natural Symptoms

Naturally infectedCotton(*Gossypium hirsutum*), exhibited severe Leaf curling, vein thickening, and enations (Fig 1). The disease incidence was significant and severity of disease symptom was high showing leaf curling, vein swelling, vein darkening and enations on the undersides of cotton leaves which frequently develop into leaf-like structures. Infected plants also showed reduced flowering.

Fig. 1: Cotton leaf showing several deformities such as vein thickening, enations etc.

Fig. B showing whiteflies on the cotton leaf.

Phylogenetic analysis

Dendrograms were used to analyse the Phylogenetic relationship between different CLCuV isolates reported from Indian subcontinent. Some other begomovirus infecting nonmalvaceous plants were also taken into account. List of all those viruses are listed in Table 1. When full length DNA-A was taken into account for similarity search, we get two broad groups, I and II. Two groups shared more than 80% nt similarity.

Two separate analyses were also done. Coat protein region (AV1) and AC1 gene were taken into account for analysis. Analysis of AV1 gene clearly indicates two groups. Interestingly, AV1 gene is the most conserve region among begomoviruses. Analysis of AC1 region indicated 4 different groups.

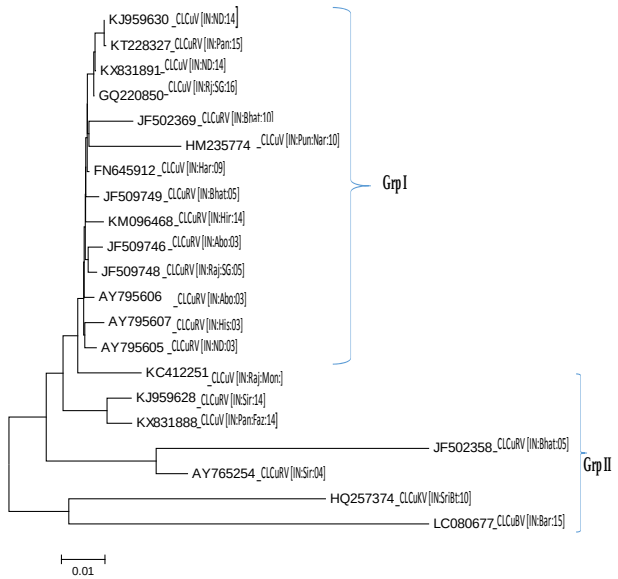

Fig. 2: The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.36222095 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 21 nucleotide sequences. Evolutionary analyses were conducted in MEGA6

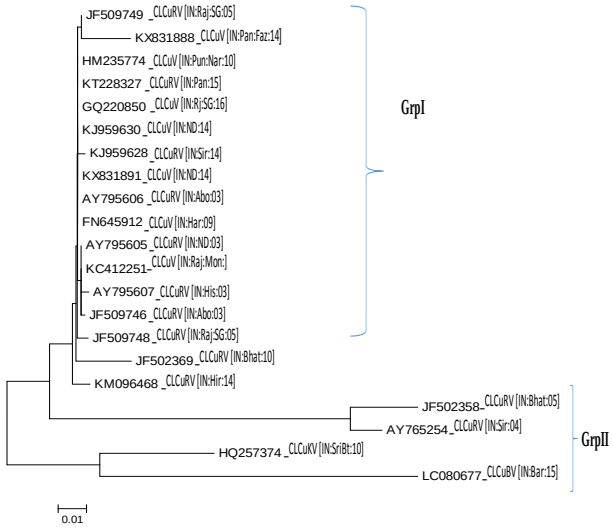

Fig3. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.40803505 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 21 nucleotide sequences. Evolutionary analyses were conducted in MEGA6.

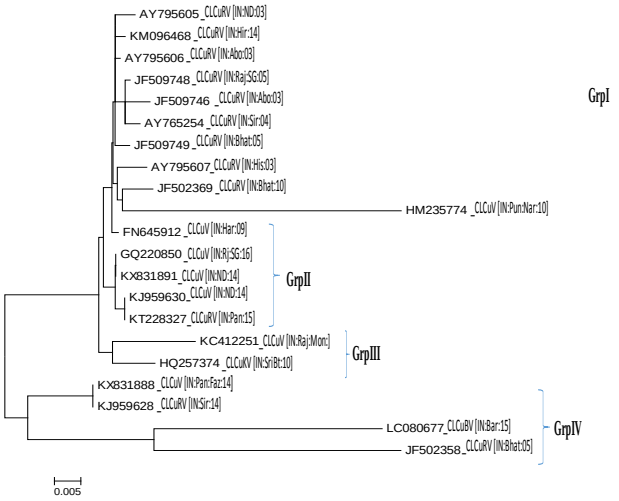

Fig. 4: The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.26847710 is shown. The tree is drawn to scale, with branch lengths in the same units

as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 21 nucleotide sequences. Evolutionary analyses were conducted in MEGA6.

4. DISCUSSION

Cotton leaf curl disease (CLCuD)-affected cotton plants develop curling of leaves, thickening of veins, enations and stunting. The most diagnostic symptom is the dark green colour of veins that is easily observed in transmitted light. There is no yield in severely affected plants, resulting in enormous losses to the growers. Until 1960s, CLCuD was confined to the Sudan and Nigeria (Varma, 1994). The Indian sub-continent was free of the disease until the late 1960s when it appeared in small pockets in the Multan area of Pakistan. The disease remained restricted to small pockets for over 20 years, but in 1988 about 60 hectares of a newly released cotton variety, S12, had a very high incidence of CLCuD, and the affected area had increased to more than 0.2 million ha by 1993. The national average cotton yield of Pakistan dropped by nearly 30%, resulting in losses of US $5 billion between 1992-97. (Briddon & Markham, 2000).

Several studies on CLCuD earlier showed that the epidemic occurring across India (Western region) and Pakistan has complex genetic structure involving several Begomovirus species (Mansoor *et al*., 2003). Molecular characterization is expected to lead to a better understanding of the taxonomy of viruses. This helps in working out how the isolate under study relates to thosereported from other parts of the world. It also sheds some light on how new strains evolve to adapt to new

hosts and under different geographical conditions. In this study, it is clear that targeting AV1 region is more appropriate to develop broad resistance against this disease. Other studies in which RNAi strategies are taken into account, this region is also appropriate.

In India, gemini viruses are widely distributed andaffects a number of plants including cotton. Till date, no single cotton transgenic has been commercialized in our country.The cotton leaf curl virus, a whitefly transmitted geminivirus (WTG), has been reported from plants other than cotton also. India has great diversity of plants, so disease spread through these whiteflies are need to be controlled, otherwise these disease complexes may prove serious threat to Indian economy.

Table 1: The name and acronym of viruses, accession number of sequences taken in this study

Virus name	Virus acronym	Accession number
Cotton leaf curl Rajasthan virus [India:Sri Ganganagar:2006]	CLCuRV	GQ220850
Cotton leaf curl virus [India:Sri Ganganagar:2016]	CLCuV	KX831891
Cotton leaf curl Rajasthan virus [India:Delhi:2014]	CLCuRV	KJ959630
Cotton leaf curl Rajasthan virus [India:Punjab:2015]	CLCuRV	KT228327
Cotton leaf curl Rajasthan virus [India:Bathinda:2005]	CLCuRV	JF509749
Cotton leaf curl Rajasthan virus [India:Hisar:2003]	CLCuRV	AY795607
Cotton leaf curl Rajasthan virus [India: Abohar:2003]	CLCuRV	AY795606
Cotton leaf curl Rajasthan virus [India: New Delhi:2003]	CLCuRV	AY795605
Cotton leaf curl Rajasthan virus [India: Abohar:2003]	CLCuRV	JF509746
Cotton leaf curl Rajasthan virus [India:Bathinda:2010]	CLCuRV	JF502369
Cotton leaf curl Rajasthan virus [India:Hisar:2014]	CLCuRV	KM096468
Cotton leaf curl virus [India:Mohanpur:2012]	CLCuV	KC412251
Cotton leaf curl Rajasthan virus [India:Sirsa:2014]	CLCuRV	KJ959628
Cotton leaf curl virus [India:Punjab:Fazilka:2014]	CLCuRV	KX831888
Cotton leaf curl virus [India:Punjab:Narauna:2010]	CLCuV	HM235774
Cotton leaf curl Kokhran virus [India:2010]	CLCuKV	HQ257374
Cotton leaf curl Barasat virus [India:Barasat:2015]	CLCuBV	LC080677
Cotton leaf curl Rajasthan virus [India:Bathinda:2005]	CLCuRV	JF502358
Cotton leaf curl Rajasthan virus [India:Sirsa:2004]	CLCuRV	AY765254
Cotton leaf curl Rajasthan virus [India:Sri Ganganagar:2005]	CLCuRV	JF509748
Cotton leaf cur Rajasthan virus [India:Haryana:2009]	CLCuRV	FN645912

REFERENCES

[1] Briddon RW and Markham PG. (2000). Cotton leaf curl virus disease. *Virus Res.*, 71: 151-159.

[2] Brown JK. (1994). Current status of *Bemisia tabaci* as a plant pest and virus vector in agro eco systems worldwide. In: FAO *Bulletein*42/1-2: 86p.

[3] Cauquil J. and Follin JC. (1983). Presumed virus and mycoplasma like organism diseases in Sub-Saharan African and the rest of the world. *Cotonet Fibres Tropicales*. 38: 293-317

[4] Cauteaux L, Lefort PL. and Kaukuvi E. (1968). Some observations on cotton leaf curl in *G. barbadense* at the Anie Mono Station, *Cotton fibr. Trop*. 23; 506-507.

[5] El-Nur E. and Abu Salih HS. (1970). Cotton leaf curl virus disease. *PANS.*16: 121-131.

[6] Estessami p, Callis R, Ellwood S. and Stanley J. (1988). Delimitation of essential genes of cassava latent virus DNA-2. *Nucl. Acids. Res*. 16: 4811-4829.

[7] Farquharson CO. (1912).*Report of a mycologist*. Annual report. Agriculture Department, Nigeria.

[8] Fauquet C, Fargette D. and Thouvenel JC. (1987). Development of African cassava mosaic virus at a Regional level in Ivory Coast. *Proceeding of the workshop of Epidemiology of plant virus diseases* (Orlando, F.L.) pp. VII-22-24.

[9] Gupta GP. (2001). Alternate strategies for insect pest management in cotton. In:*Alternate Strategies for insect Pest Management in Major Crops* (Ed. V.K. Sehgal). Division of Entomology, IARI, New Delhi, 232-236 p.

[10] Hussain T. and Ali M. (1975). A review of cotton diseases of Pakistan. *The Pakistan Cotton*, 19: 71-86.

[11] Mansoor S, Mukhtar S, Hussain M, Amin I, Zafar Y and Malik KA. (2000). Widespread occurrence of cotton leaf curl virus on radish in Pakistan*. Plant Disease*,84: 809.

[12] Narula AM, Monga D, Chauhan MS. and Sheo Raj. (1999). Cotton leaf curl virus disease in India. The challenge ahead*. J. Cotton Res. Dev.*13(2): 129-138.

[13] Noueiry AO, Lucas WJ. and Gilbertson RL. (1994). Two proteins of a plant DNA virus coordinate nuclear and plasmodesmatal transport. *Cell* 76: 925-932.

[14] Rogers SG, Bisaro DM, Horsch RB, Fraley RT, Hoffman NL, Brand L, Elmer JS. and Lloyd AM. (1986). Tomato golden mosaic virus A component DNA replicates autonomously in transgenic plants. *Cell*, 45, 593-600.

[15] Sunter G, Gardiner WE, Rushing AE, Rogers SG. andBisaro DM. (1987). Independent encapsidation of tomato golden mosaic virus A component DNA in transgenic plants. *Plant Mol. Biol*. 8, 477.

[16] Townsend R, Watts J. and Stanley J. (1986).Syntheis of viral DNA forms in *Nicotianaplumbaginifolia*protoplasts inoculated with cassava latent virus (CLV): Evidence for the independent replication of one component of the CLV genome. *Nucl. Acids Res*. 14: 1253-1265.

[17] Varma A. (1994).*Leaf curl Disease of Cotton in North West India.* Report of the ICAR committee. September, 1994.