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COTTON LEAF CURL VIRUS DISEASE COMPLEX - A REVIEW

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Cotton leaf curl disease has become the major limitation in the production of cotton, which is an important fibre and cash crop of India and has spread into almost all the cotton growing belts. During 1997-1998 seasons, a sudden flare up of this disease was noticed in three states viz., Rajasthan, Punjab and Haryana. The characteristic symptoms include upward leaf curling, vein thickening following dark green discoloration and leaf like structure called enations on the reverse side of leaves. The importance of this disease stems from the fact that it is responsible for losses to the tune of 60 percent. Cotton leaf curl disease is transmitted by the whitefly, *Bemisia tabaci* in persistent manner. This disease is caused by a complex consisting of the monopartite begomovirus cotton leaf curl virus (CLCuV) of the family *Geminiviridae* and a nonovirus like component. The etiology of cotton leaf curl disease has shown to be uncertain and complex.

Keywords : Begomovirus; *Bemisia tabaci;* Cotton leaf curl virus; Gossypium *hirsutum;* Nonovirus; *Whitefly.*

Introduction

Cotton is one of the most important crop amongst fibre and cash crops of India, accounting for over 30% of the countries foreign exchange. *Gossypium hirsutum* is most widely grown and contributes about 80% of the total production. Cotton can be grown nearly throughout the year (ratoon), since climate conditions conducive to its growth are available in one or other part of the country. In spite of having largest area (9.25 million ha) under cotton in the world, Indian share is only 1/10th of the world production with a total production of 321 kg/ha[1]. Cotton leaf curl disease (CLCud), earlier known as African leaf curl of cotton was first noticed in Nigeria on *G. peruvianum* and *G. vitifolia*[2]. Later serious outbreak of this disease was recorded in Nigeria[3]. Subsequently this disease appeared in Sudan, Tanzania[4,5,6], Pakistan[7] and India[8,9,10]. Low incidence of CLCuV-K was also reported from south India in *G. barbadense*[11]. The disease was first noticed on few *G. barbadense* plants in 1989 from New Delhi. CLCuD came in prominence in 1993 when a few patches were affected in a block of a newly released variety of cotton (F-846) near Sriganganagar in Rajasthan. The disease continued to spread rapidly and the area affected was 500 ha in 1994-95 and exceeded 10,946 ha in 1996. In Punjab state, disease was noticed in an area of 1500 ha during 1994. In 1997 its outbreak took place and about 80,000 ha area was found affected with this disease. In the Haryana state, situation was better till 1996 where disease was limited to about 20 ha. During 1997-98 season, a sudden flare up of this disease in all the three states and as per estimates an area of about 2.20 lakh ha was infested due to CLCuD. This disease continued to spread until it occurred throughout the cotton growing area of the northern India. During early phase of epidemic, the disease spread rapidly to the northern parts, followed by the strong prevailing winds. In Pakistan, this disease occurred in epidemic proportion in 1992-93 and 1993-94 affecting 889,000 ha and spread both south into the Sindh region and across the border in north western India. The reduction in yield due to the CLCuD incidence depends largely on the varieties grown, time of infection and severity of disease. The losses to the Pakistan foreign exchange are estimated US $ 5 billion between 1992 to 1997.

The first report of tobacco whitefly, *Bemisia tabaci* (Gennaduis) transmission of this disease was reported by various workers[4,12]. This disease is graft transmissible but not by seed and mechanical inoculation[13,14,15,16]. Single insects could then transmit CLCuD from infected to healthy cotton plants. There are very few reports on the virus-vector relationship[11,17] and based on field observations, it was noticed that if

the disease appeared at late stage when the plants were aged and probably mature enough to tolerate the attack, the crop gave good yield even in the presence of the leaf curl disease. However, when most of the crop was attacked at young stage it suffered severely resulting in almost complete loss of the crop. The sowing dates are another important factor which play a decisive role in disease incidence as well as insect population than late sown (3dr week of May) cotton. The progress of disease in general was maximum during the month of August as compared to July and September/October. Whiteflies are usually a problem in the mid to late season (August to October) and a significant positive correlation between the per cent disease incidence and whitefly count was established[18]. The data indicated that CLCuD is not seed-borne, both the disease and insect vector must survive on reservoir hosts for further inoculum's spread. The experimental host range of CLCuD includes cotton, tobacco, tomato, chinarose, ageratum, okra, French bean and hollyhock. In the fields, leaf curl like symptoms have been observed on many herbaceous and woody species like okra, sunflower, chinarose and many weeds. Another observation has revealed that the disease was more near the orchards more often in one direction of the orchard and not the other. Interestingly, no disease appears on the desi cotton, *G. arboreum* so far. Probably, the initial source of infection may be weeds or the surviving ratoon cotton, infected during previous season. Cotton varieties being used in north-west India have played a major role in the epidemic. The varieties like F 846, RST 9 and HS2 are now known to be more susceptible to CLCuD. As per the experience in the country and also elsewhere in the world, the consensus was that CLCuD problem could be managed most effectively by evolving and introducing resistant/ tolerant varieties.

In India, breeding programme involving resistant varieties is also being undertaken at various institutes and has developed resistant varieties including RS 875, LRA 5166 and LH 144. In the coming years, the areas affected by leaf curl disease shall have to be either replaced by arboreum cottons or resistant hirsutum materials as has been successfully achieved in case of countries where leaf curl has remained problem.

Etiology of the disease

The infected cotton plants exhibit upward curling of leaves, which occurs because of the uneven growth of veinal tissues on the reverse side of leaves (Fig. la). Veins of the leaves become thickned with enations on the underside of leaves, which frequently develop into leaf like structures. Affected leaves become dark green than healthy leaves. Plants effected at early stage leads to reduction in internodal length, stunting and less flowering.

Recently cotton leaf curl disease has attracted attention and showed symptoms typical of those caused by whitefly-transmitted geminiviruses. Earlier evidence of the association of whitefly-transmitted begomoviruses with many of these diseases was obtained by using serological[11,19] and polymerase chain reaction (PCR) based techniques[19,20,21,22]. However, none of these reports provided clear evidence on the causative agent of the disease.

Association of cotton leaf curl disease and Begomovirus - Members of the family *Geminiviridae* are a group of plant viruses that have small twinisometric particles containing a single-stranded DNA genome (Fig 1b,c). Geminiviruses are classified into four genera based on their hosts, insect vector and genome organization. Viruses in the genus Mastrevirus infect mainly monocots, have monopartite genomes and are transmitted by leafhoppers. Viruses in the genus Curtovirus enfect dicotyledonous plants, have monopartite genome and are transmitted by leafhoppers while viruses of Topucovirus genus are transmitted by

treehopper, which have monopartite genome and infect dicot plants. Most of the economically important geminiviruses are in the genus Begomovirus, which consists of more than 100 species and are transmitted by the whitefly, and infect dicotyledonous plants. Although the majority of begomoviruses have bipartite genomes but an increasing number of them are being identified that have only a single component of the bipartite viruses. CLCuV-Pak1 and TYLCV-Is the most notable and economically most significant examples of monopartite begomoviruses[23,24]. Studies on leaf curl disease revealed that it is transmitted by the whitefly, *B. tabaci*, which led to believe that a begomovirus might cause the disease. PCR based observations showed the presence of a begomovirus in CLCuD infected plants[20,25,26]. Therefore the begomovirus associated with leaf curl disease of cotton is caused by cotton leaf curl virus (CLCuV). Various groups established the diversity of CLCuV in Pakistan and India[27,28,29]. However these investigations did not attempted infectivity studies with their cloned genome. Efforts to identify a second genomic component were unsuccessful[30,31,32]. The cloned genome of CLCuV was infectious to cotton and *Nicotoina benthamianum* but did not induce symptoms typical of cotton leaf curl disease[32]. These findings suggest that the CLCuV is not the only causative agent of cotton leaf curl disease. The genome of CLCuV, equivalent to the DNA component of bipartite begomoviruses has feature typical of the old world geminiviruses, having AV2 gene (Fig 1c). Phylogenetic analysis based upon alignments of the sequences of the coat protein gene of CLCuV with the other begomoviruses (Fig 2). The dendrogram shows that CLCuV is the most closely related to begomoviruses originating from the Indian sub-continent (old world).

Association of cotton leaf curl disease and a nonovirus like molecule- Recent work has revealed another type of circular single stranded DNA satellite molecule, known as β DNA, associated with all the leaf infected cotton plants that were tested from several locations of Pakistan and India[25,30,33]. This β DNA molecule is approximately 1350 nucleotide long and encodes a replication associated protein (Rep) of nonovirus. This satellite molecule is capable of self-replicating in plant cells and is encapsidated by the CLCuV coat protein when co-infected with begomovirus. Despite the diversity of begomoviruses associated with CLCuD, only a single class of DNA β has been detected, suggesting that satellite has the capacity to be recruited by unrelated begomoviruses[34]. The nonoviruses are a genus of plant infecting multicomponent single stranded DNA viruses that are transmitted in a circulative manner by aphids or planthoppers. In contrast, the geminiviruses are transmitted in circulative manner either by leafhoppers, whitefly; *B. tabaci* or treehoppers. Although nonoviruses have not been studied in a much details as geminiviruses but these viruses have several features common with geminiviruses that suggest their replication mechanisms are very similar. Each of nonovirus DNA species has a common region that 5- TANTTATTAC-3 which is found in the origin of geminiviruses (+) strand synthesis[35]. A similar DNA β molecule has been detected in *Ageratum conyzoides* plants originating from Singapore, exhibiting yellow vein symptoms and caused by a monopartite begomovirus[36].

Discussion

Cotton leaf curl disease has attained a serious status in cotton growing areas of northern India. There is strong probability that CLCuD has come across the border in the states of Rajasthan and Punjab through the migrating whitefly, *B. tabaci* which is an efficient vector of the geminiviruses. Information on appearance and incidence of leaf curl variants of cotton from different locales of India will be useful to play suitable measure to check this disease and for

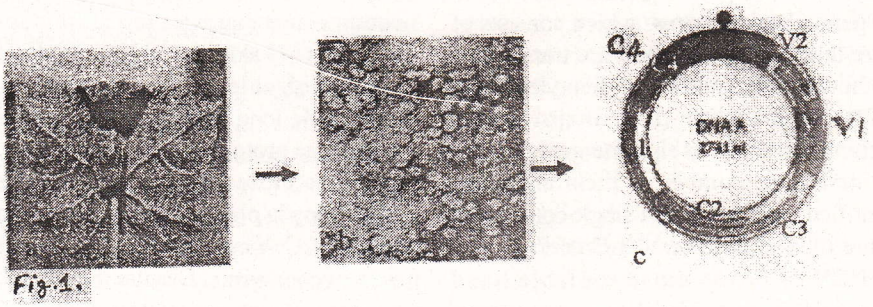

Fig 1. a) Leaf curl symptoms showing vein thickening, upward curling and enations on the reverse side of leaf; b) Electron microscope showing twin shaped particles and c) Genome map of cotton leaf curl disease.

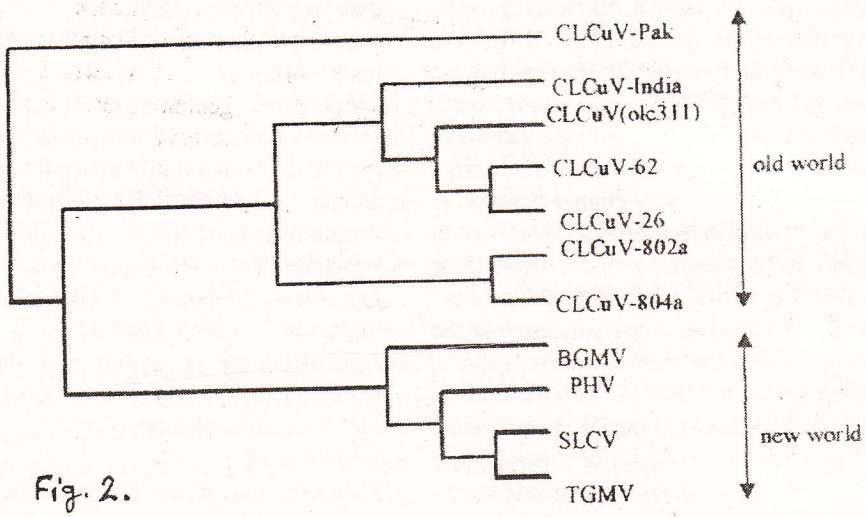

Fig 2. Phylogenetic dendrogram based on alignment of the complete amino acid sequences of the CP region of cotton leaf curl virus and other selected geminiviruses. The geographic origins of the viruses are indicated on the right. Virus sequences are obtained from the EMBL sequence database. The viruses are; cotton leaf curl virus (CLCuV), Bean golden mosaic virus (BGMV), Physalis mottle virus (PHV), Squash leaf curl virus (SLCV) and Tomato golden mosaic virus (TGMV).

monitoring cotton productivity. Cotton leaf curl disease reported from north India has similar symptoms as that of New Delhi and southern part of India. In fact, it is not possible to rule out the accidental introduction of this disease from North West India into these areas. Therefore, it seems most likely that these diseases are unrelated and is an uncharacterized disease, similar to cotton leaf crumple virus which was also transmitted by the whitefly[37]. Therefore, a detailed molecular analysis is required to establish any relationship between viral diseases of cotton. Realizing the potential threat of leaf curl disease of cotton, it is feared that in future Indian cotton growing areas might be as badly affected by this disease as happened in Pakistan. Looking at the gravity of the situation, urgent step should be taken for long term management of the disease. Therefore information regarding molecular characterization of CLCuV is prerequisite to know the exact causal agent of the disease. Cotton leaf curl disease is known to be transmitted by the whitefly, *B. tabaci* in persistent manner suggest that this belong to a geminivirus group. More recently, scientists have established cotton leaf curl disease identity as the genus *Begomovirus* of the family *Geminiviridae*. Etiology of the disease is still shown to be complex. Despite efforts are underway to identify both begomovirus and a nonovirus like component association with leaf curl disease of cotton.

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