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A WHITEFLY-TRANSMITTED GEMINIVIRUS ASSOCIATED WITH COTTON LEAF CURL DISEASE IN PAKISTAN

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Cotton (*Gossypium hirsutum* L.) is one of the most important crop of Pakistan which accounts for 60% of the export product of the country. In the last few years cotton leaf curl disease has acquired epidemic proportions in Pakistan and has seriously threatened cotton production. The characteristic symptoms of the disease are severe leaf curling, thick dark veins and enations which sometimes differentiate into cup shaped leaf-like structures on the underside of the leaf (Fig 1a).

Whitefly (*Bemisia tabaci*) was suspected as the insect vector of cotton leaf curl disease. Whiteflies maintained in the controlled conditions were used for insect transmission of the disease from cotton to cotton and from cotton to tobacco (*Nicotiana tabacum*). The symptoms developed on cotton and tobacco were similar to leaf curling in cotton plant with thick dark veins and development of cup shaped structures on underside of leaf (Fig. 1b).

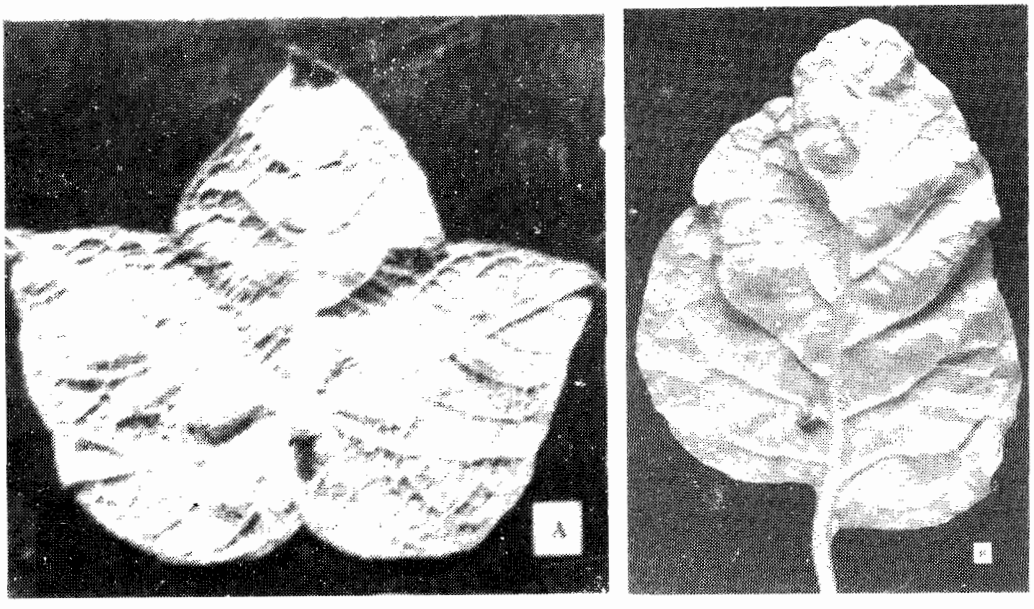

Fig 1. Symptoms of cotton leaf curl disease. A. typical symptoms on cotton leaf. B. symptoms on tobacco after whitefly transmission of the disease

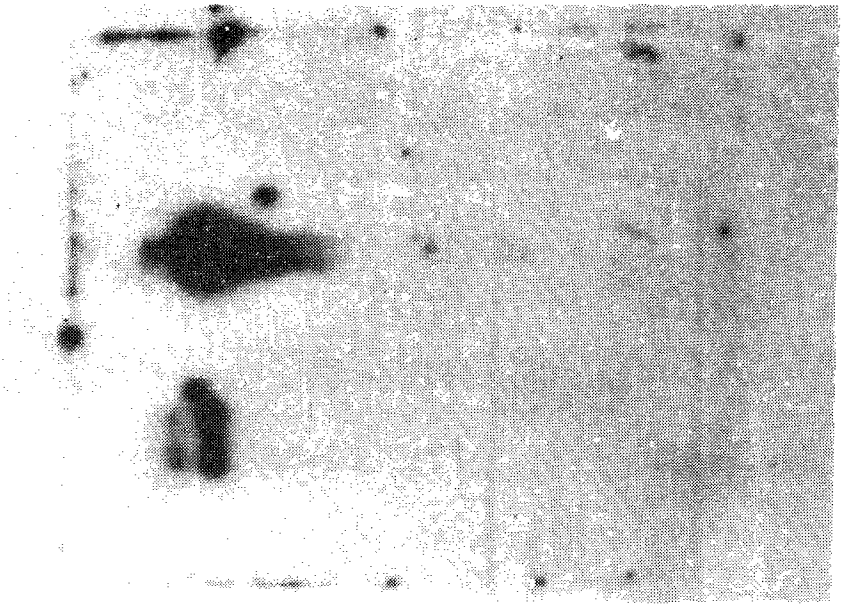

Fig. 2. Southern hybridization of of total nucleic acid with ACMV DNA A probe. lane 1, total nucleicacid isolated from tobacco infected with ICMV. Lane 2 and 3, total nucleic acid isolated from CoLCV infected tobacco.

There are several groups of viruses transmitted by whiteflies. One of the most important group of these viruses is the geminivirus group (Harrison, 1985). Geminivirus has circular single-stranded DNA genome encapsidated in twinned (geminate) particles (Stanley, 1991). Cotton leaf curl virus seems to belong to this group of viruses.

To determine the nature of virus, total nucleic acids were isolated from healthy and infected tobacco plants and subjected to agarose gel electrophoresis and blotted onto nylon membrane (Hybond N, Amersham, UK) as described by Stanley *et. al.*, (1990). P^{32} labelled DNA A of African cassava mosaic virus (ACMV) and Indian cassava mosaic virus (ICMV) was used as probe to detect geminivirus DNA. The ACMV and ICMV DNA A cross hybridized with cotton leaf curl virus (CoLCV), suggesting that CoLCV might be a geminivirus. Single stranded (SS)and double stranded supercoiled replicative form (CCC) associated with geminiviruses could be identified on Southern hybridization (Fig. 2).

Geminiviruses are known to replicate via a double stranded circular DNA intermediate-the replicative form which can serve as template for amplification by polymerase chain reaction (Rajos *et al*, 1993). Universal PCR primers for geminiviruses based on conserved C1 gene involved in viral replication have been designed (Briddon *et al*, personnal communication). The use of these primers gives full length geminivirus DNA. Using these primers we were able to amplify DNA of size (2.8 kb) expected for geminiviruses. These primers were successfully used to amplify DNA from cotton, and tobacco of the expected size (Fig. 3). The amplification from cotton and tobacco where virus was transmitted from cotton gave an additional product of 1.7 kb which is suspected

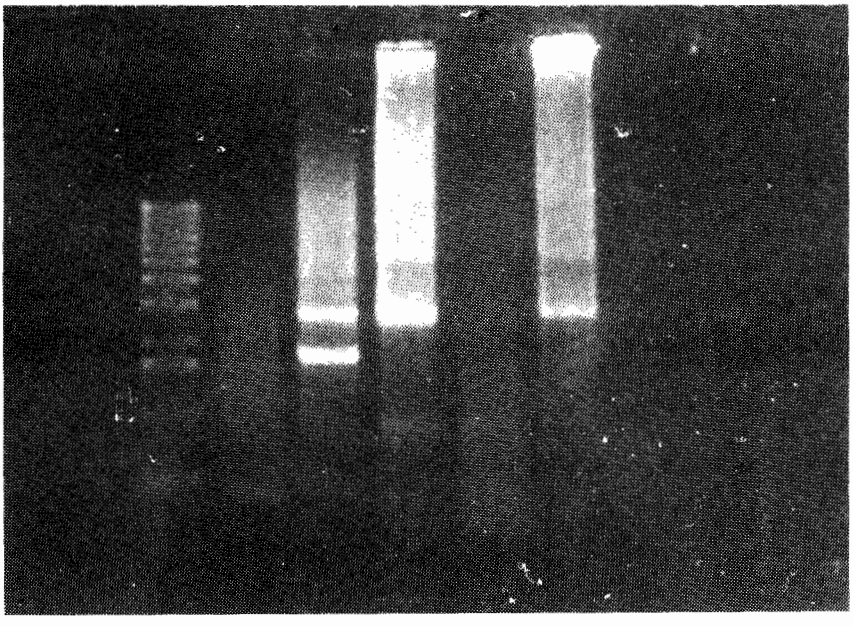

Fig. 3. PCR amplication of geminivirus DNA with PCR primers based on C1 gene. Lane 1 DNA size markers, Lane 2 healthy tobacco, Lane 3 CoLCV , Lane 4 ICMV, Lane 5 healthy N. benthamiana, Lane 6 ACMV.

to be subgenomic DNA. PCR amplified DNA hybridized with ACMV DNA A probe, suggesting that PCR amplified the geminivirus DNA. The amplified subgenomic DNA also hybridized with the probe.

Our data shows that cotton leaf curl disease is associated with a geminivirus, which is transmitted by whiteflies. Similar disease transmitted by whiteflies was described from Sudan in 1960's, where the pathogen was not fully characterized. It is therefore difficult to compare CoLCV with the disease in Sudan. Efforts are going on to obtain infectious clones of CoLCV virus as described earlier for other geminiviruses (Stanley, 1983). The agroinoculation of the infectious clone will prove this virus as the causative agent of this disease.

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