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# Efficiency of *Bemisia tabaci* (Gennadius) populations from different plant-hosts for acquisition and transmission of cotton leaf curl virus

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Whitefly (*Bemisia tabaci*) populations collected from five crop plants, viz brinjal, cotton, potato, tomato, soybean and a weed, *Sida* sp., and maintained on respective host plants were studied for virus acquisition from diseased cotton plant and its subsequent transmission to healthy cotton plants. The presence of virus in whitefly and diseased plants was established by PCR amplification of CLCuV-specific DNA primers ($P_{1800-500}$). The virus acquisition from the diseased cotton plants as well as its transmission to healthy cotton plant to cause disease was 100 per cent in the case of cotton-specific whitefly. Compared to this, using whitefly from other host-plants, both the values for acquisition of virus as well as its subsequent transmission decreased, being higher with soybean-specific whitefly (80 and 70%, respectively) and minimum with tomato whitefly (20% each). However, the actual virus transmission efficiency (ATE), when expressed after taking into account the fraction of whitefly samples that did not acquire virus, though was found to be 100 per cent in the case of cotton-, tomato- and brinjal-whitefly, it remained more than 67% with whitefly from other crops/weed. The study revealed that whitefly populations that are specific to different host-plants, differ primarily in their efficiency for acquiring CLCuV and once the virus is acquired it is efficiently transmitted to healthy cotton plants to cause cotton leaf curl disease (CLCuD). The results hold significance in control of CLCuD through appropriate management of whitefly on alternate host plants/weeds, both in and around the cotton growing areas.

**Keywords:** *Bemisia tabaci,* plant hosts, CLCuV transmission, whitefly

## Introduction

Begomoviruses (Family *Geminiviridae,* Genus *Begomovirus)* are assumed to have been co-evolving with their dicotyledonous plant hosts for a long time[1]. In the past two decades, agricultural intensification has resulted in begomovirus disease outbreaks in tropical and subtropical regions, causing devastating yield losses of many crops[2-4]. Cotton leaf curl viruses (CLCuV) - a group of begomoviruses has emerged as a serious threat to cotton production especially american cotton cultivars (*Gossypium hirsutum* L.), which accounts for 85% of global lint production. In Indian subcontinent, cotton leaf curl disease (CLCuD) was first recorded from Rajasthan during 1993 and to date this has spread to the entire cotton belt of North-western India[5]. In 2004, outbreak of CLCuD in this region accompanied by a disease incidence ranging from traces to 100% and location mean ranging from 4.9 to 57.0% was observed[6]. Though G. *hirsutum* is the natural host for CLCuV, many species of Malvaceae and Solanaceae have also been found to be alternate experimental hosts for this virus[7]. Like other begomoviruses, transmission of CLCuVs is also vectored by whitefly, *Bemisia tabaci* (Gennadius), which is a pest of over 600 host plants including vegetables, fibre crops, spices, ornamental plants and many species of weeds[8,9]. Host plant pressure is known to exert a direct effect on the morphology, development, fecundity and survival of *B. tabaci*[10-12]. In cotton belt of North-western India as well, *B. tabaci* has been found to remain active on many host crops (brinjal, chillies, cucurbits, cotton, mentha, okra, potato, rapeseed, sunflower and tomato, etc.) and weeds *(Sida* sp, *Abutilon indicum* and *Althea rosea)* throughout the year[5]. Several studies suggested the existence of diverse populations of *B. tabaci* that vary to some extent in their capacity to develop high population densities and cause direct feeding damage in different hosts. This may indirectly reflect on the host range of the virus and the efficacy with which they can be transmitted[13,14]. Thus, persistence of the whitefly pest on a wide range of hosts throughout the year is likely to influence persistence and

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transmission of CLCuVs. Using RAPD-PCR markers, we have earlier demonstrated the existence of host-plant specific genotypes in *B. tabaci*[15]. Since existence of molecular differences amongst different genotypes of whitefly is likely to have broad influence on its CLCuV vectoring efficiency, *B. tabaci* populations holding specificity for different host plants were investigated for their capacity to acquire CLCuVs from diseased cotton plants and its subsequent transmission to healthy cotton plants.

## Materials and Methods

### Whitefly Source

Whitefly populations that have earlier shown specificity to five different host plants (4 cotton, brinjal, potato, tomato and soybean) and a weed (*Sida* sp.)[15] were isolated and maintained on respective host plants in separate screen cages.

### Virus Acquisition and Transmission

A specific whitefly population was transferred into leaf cages (12 individuals/cage) that were fixed on leaves of a diseased cotton plant (*G. hirsutum* 348) kept in a separate screen cage. After 24 h of optimal acquisition access period[16], all the individuals were recollected and pooled. Total DNA from 10 whitefly individuals was analyzed for the presence of CLCuV-DNA using CLCuV specific PCR-amplification. Parallel to this, another sample of 10 whitefly individuals was transferred to 10 healthy cotton plants (at 4-leaf stage) that were grown separately under glass *chimnies* for observations on transmission of CLCuV and CLCuD symptoms. After 24 h of optimal transmission period[16] all the whiteflies were retrieved, growth of individual plants continued under insect and virus free conditions. The plants were monitored up to 14 d for development of typical symptoms of CLCuD i.e., thickening of veins, upward curling of leaves along the margins and enations on underside of leaves[17]. The whitefly individuals retrieved from cotton plants after virus transmission were also analyzed for CLCuV by PCR amplification.

### Molecular Detection of CLCuV-DNA in Whitefly and Plant DNA

For molecular detection of virus in whitefly and diseased plants, CLCuV specific primer set $P_{1800\text{-}500}$ ($F_{1800}$: CCTCCTTTAATTTGAACCGG; $R_{500}$: GGCTTTCTGTACATGGGCCTGT) were used for PCR-amplification of a CLCuV-specific DNA fragment (~1500 bp) from whitefly and plant DNA templates[18]. The coordinates of these primers in the DNA of different begomoviruses are given in Table 1.

### DNA Extraction

Total DNA from a whitefly individual and diseased cotton plant leaf (~1 g) was isolated by standard methods[19,20]. These methods provided 50 µL of PCR-grade DNA solution ( ~3 ng DNA/µL) from whitefly and 500 µL of cotton DNA (50 ng/µL). The DNA concentration was quantified by $A_{260}/A_{280}$ ratio in a Biophotometer.

### PCR for CLCuV- DNA

For CLCuV specific-PCR amplifications, the PCR reaction was performed in 25 µL reaction mixture, which contained 1 m*M* dNTPs mix (3 µL), 5 ng/µL forward and reverse primers (2.5 µL each), 2.0 units Taq Polymerase (MBI, Fermentas), 2 µL DNA solution from whitefly (30 ng) or cotton leaf (100 ng), 10 × Taq reaction buffer (2.5 µL) and sterile dd $H_2O$ to make 25 µL. The PCR program utilized following thermal profile: 95°C-5 min (preheating); 94°C-1 min, 52°C-1 min and 72°C-2 min (28 cycles), followed by 72°C-10 min as final elongation step and 4°C for storage until use. The amplified product was resolved in 0.7% Agarose gel (in Tris-Acetate-EDTA buffer) by horizontal gel electrophoresis. The presence of CLCuV specific band in amplified product lanes was observed in ethidium bromide stained gel on a UV transilluminator and photographed with a Gel Documentation system. The

Table 1—Coordinates of the CLCuV specific primers in the reported sequences of different begomoviruses in GenBank

| Name of begomovirus | GenBank Acc No. | Coordinates of primers (5′→3′) in the GenBank sequence | |
|---|---|---|---|
| | | F1800 | R500 |
| Cotton leaf curl Sri Ganganagar virus | GQ220850 | 1713-1732 | 446-425 |
| Cotton leaf curl Multan virus | FJ218487 | 1703-1722 | 438-417 |
| Cotton leaf curl Gezira virus | FJ469627 | 1745-1764 | 481-460 |
| Okra leaf curl Mali virus | EU024120 | 1746-1765 | 482-461 |
| Cotton leaf crumple virus | AY742220 | 1578-1597 | 336-315 |
| Cotton leaf crumple virus | AF480940 | 1538-1602 | 337-316 |
| Okra yellow mosaic Mexico virus | EF591629 | 2058-2078 | 813-792 |

size of individual bands in the amplified profile was determined by comparison with a co-migrating DNA ladder (100 bp DNA ladder plus, MBI Fermentas).

## Results and Discussion

The vectoring efficiency of *B. tabaci* for disease transmission is a combined function of two phenomenon i.e., acquisition of virus from diseased plants by the vector/carrier whitefly; and the transfer of the acquired virus by whitefly into healthy cotton plants. In the infected plants, the virus DNA multiplies and subsequent expression of viral genes results in the appearance of disease symptoms. Therefore, in order to establish the efficiency of virus acquisition, the experiments were performed using whitefly individuals, and acquisition of virus from a diseased cotton plant was detected by PCR amplification of CLCuV specific DNA from a sample of 10 whitefly individuals (Fig. 1). Amplification of CLCuV-DNA specific fragments (~1500 bp) established that in the case of cotton specific whitefly population, all the whitefly individuals had acquired virus from the diseased cotton plant, thus showing a virus acquisition efficiency of 100%. Compared to this, whitefly populations from all the other plant hosts showed a reduced efficiency of virus acquisition, with maximum in whitefly from soybean (80%) and minimum in tomato whitefly (20%) (Fig. 1, Table 2). Thus, cotton whitefly being a cotton specific genotypes[20] appeared to be optimally adapted to virus acquisition from diseased cotton plant. However, decreased efficiencies for virus acquisition in whiteflies from other alternate host plants may be related to different whitefly genotype[15] with low adaptability to cotton resulting from a residual alternate host plant effects[21], and retarded development on infected cotton plants[22].

During subsequent virus transmission studies to healthy cotton plants, only cotton whitefly induced CLCuD symptoms in all the 10 plants studied (Table 3). This 100% observed transmission efficiency (OTE) was supported by specific amplification of CLCuV-DNA from all the infected cotton plants (Table 3, Fig. 2). In the case of whitefly holding specificity to other host plants, the OTE values varied between 20% (tomato) to 70% (soybean). PCR analysis of whitefly individuals that were retrieved after viral transmission suggested that in the case of cotton, tomato and brinjal whiteflies, all the whitefly individuals with acquired virus had actively transmitted the virus to cause CLCuD. However, a minor fraction of such individuals from potato (2 out of 6), soybean (1 out of 8) and *Sida* sp. (1 out of 5) failed to transmit the same to cotton. It was specific to note that CLCuD symptoms were observed in only those cotton plants in which presence of CLCuV was established by PCR, others remained healthy.

The above expression of OTE did not take into account the proportion of whitefly individuals that had acquired virus. In case of cotton whitefly, all the individuals carried the virus and hence all of these participated in further transmission of virus. However, in case of whitefly individuals holding specificity to other alternate hosts, a sizable number of individuals (10 each) though participated in virus transmission but did not carry the acquired virus. For example, in the case of soybean whitefly, only seven out of ten

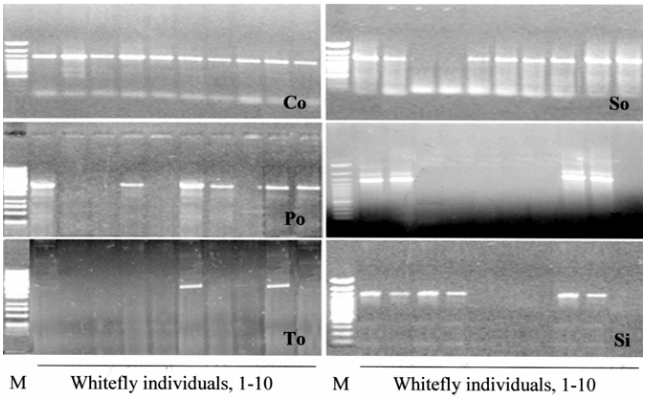


Fig. 1—PCR amplification of CLCuV-DNA in individuals of different whitefly types (plant hosts) after virus acquisition from infected cotton plants. Co-Cotton, So-Soybean, Po-Potato, Br-Brinjal, To-Tomato, Si-*Sida* sp., M-100 bp DNA size marker lane (MBI Fermentas).

Table 2—Detection of CLCuV DNA by PCR amplification in whitefly individuals after virus acquisition

| Host specificity of whitefly | No. of whitefly individuals showing amplification of CLCuV DNA* | CLCuV acqusition efficiency (%) |
|---|---|---|
| Cotton | 10 | 100 |
| Potato | 6 | 60 |
| Tomato | 2 | 20 |
| Soybean | 8 | 80 |
| Brinjal | 4 | 40 |
| *Sida* sp. | 6 | 60 |

*10 whitefly individuals drawn from each whitefly sample were PCR amplified for CLCuV specific amplification.

Table 3—CLCuV transmission efficiencies of whitefly from different host plants

| Host specificity of whitefly | CLCuV transmission | | CLCuV transmission efficiency (%) | | No. of retrieved † whitefly individuals with CLCuV DNA |
|---|---|---|---|---|---|
| | PCR reaction* | Disease symptom** | Observed (OTE) | Actual (ATE) | |
| Cotton | 10 | 10 | 100 | 100 | 10 |
| Potato | 4 | 4 | 40 | 67 | 6 |
| Tomato | 2 | 2 | 20 | 100 | 2 |
| Soybean | 7 | 7 | 70 | 88 | 8 |
| Brinjal | 4 | 4 | 40 | 100 | 4 |
| *Sida* sp. | 5 | 5 | 50 | 83 | 6 |

*Per 10 whitefly individuals each one retrieved from respective cages fixed on separate plants; **Per 10 cotton plants; † Out of 10 whitefly individuals retrieved from cotton plants after virus transmission.
OTE = Transmission as established by PCR reaction & disease symptoms/number of whitefly used for transmission × 100
ATE = observed transmission efficiency / acquisition efficiency × 100

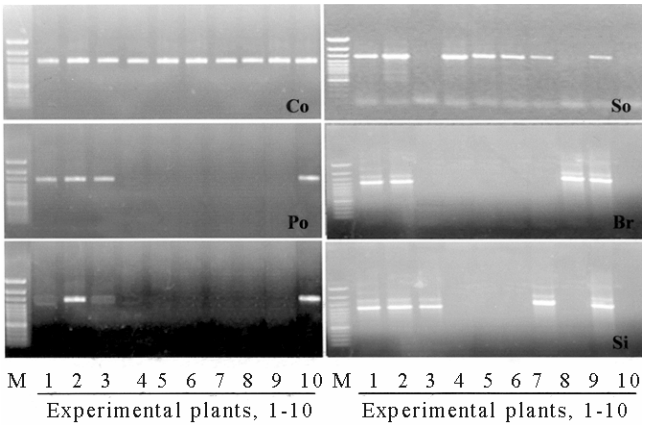


Fig. 2—PCR amplification of transmitted CLCuV-DNA in cotton plants by whitefly from different plant hosts. Co-Cotton, So-Soybean, Po-Potato, Br-Brinjal, To-Tomato, Si-*Sida* sp, M- M-100 bp DNA size marker lane (MBI Fermentas).

whitefly individuals carried the virus and all of these caused effective transmission of virus to cause disease in only seven out of ten plants tested. Considering this factor, the 'Actual transmission efficiencies' (ATE) were calculated based upon the fraction of whitefly individuals that carried the acquired virus in a population of 10 whitefly individuals used for virus transmission studies. On this basis all the tomato and brinjal whitefly individuals (2 & 4, respectively), which acquired the virus had resulted in appearance of disease symptoms and amplification of virus DNA in an equal number of cotton plants, also expressed 100% ATE. This was followed by whitefly type specific to soybean (87.5%), *Sida* sp. (83.3%) and potato (66.7%). These results suggested that the whitefly populations from different host plants differed greatly in their capacity to acquire CLCuV from diseased cotton plants. However, once acquired the virus is actively transmitted to cotton plants. The results also reveal that the differences in virus transmission efficiency of different whitefly populations are primarily due to differences in acquisition of virus, though they may differ with respect to virus transmission efficiency as well. Besides above, the decreased transmission efficiencies can also be attributed to decline in virus transmissibility upon maintenance of whitefly with acquired geminivirus on alternate host plants[23].

The differences in virus acquisition efficiency amongst whitefly populations from different host plants could be due to some unknown biochemical functions related to genetic differences amongst these whitefly types or the host effect[24]. The differing composition (amino acid composition) of sap solutions from different host plants are known to influence the growth and development of sap sucking insects[25]. Thus, it is also possible that some unknown factors in sap solutions used by the infesting whitefly during its growth on alternate host plants might be interfering with the subsequent acquisition of virus from diseased cotton plants[25]. Similarly, it may also be possible that availability of certain cotton factors might be involved/inductive to active acquisition of virus by cotton whitefly and its transmission to cause disease in healthy cotton plants.

Different host crops are known to exert their influence by selecting specific whitefly genotypes out of several, which exist in a particular agroclimatic region[20]. Such a scenario might have resulted in differential behaviour of acquisition and transmittance of CLCuV due to influence of host selection

pressure[26,27]. These studies assume significance in the wake of evidence that whitefly from different host crop show genetic variability[20]. The studies also revealed that since soybean, tomato and brinjal are grown in *kharif* season along with cotton, cultivation of these crops in the neighbourhood or around the cotton fields can have a devastating influence on occurrence of CLCuD in cotton fields due to active transmission of CLCuV by the whitefly populations prevailing in the fields of these alternate host crops. Similarly, potato acts as an alternate host in the off-season and helps in preserving the CLCuV inoculum, which can be effectively transmitted to other cultivated crops growing in the vicinity. The findings stress upon the proper management of alternate host crops/weeds in the off-season as well as during crop season for controlling the transmission of CLCuV in cotton crop.

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