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Relationship of Cotton Leaf Curl Virus Symptoms with Virus Concentration and Epitope Profile

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Abstract

Vein thickening, enation formation and curling of leaves (upward or downward) are characteristic symptoms of cotton leaf curl virus, a whitefly (*Bemisia tabaci*) transmitted geminivirus, in cotton. To ascertain the effect of virus titer on type of curling and their relationship with epitope profile we determined virus concentration in 26 samples (13 each upward and downward curling) and established epitope profile of 34 samples (17 each) by TAS-ELISA using polyclonal antisera to Indian Cassava Mosaic Virus (ICMV) and Monoclonal Antibodies (MAbs) to African Cassava Mosaic Virus (ACMC) & ICMV and Okra Leaf Curl Virus (OLCV). No relationship between virus concentration and epitope profile was found with type of symptoms.

Introduction

Leaf curl disease of cotton (*Gossypium hirsutum L.*) caused heavy economic losses during the epidemic year of 1992-93. The disease is caused by a whitefly (*Bemisia tabaci* Gennadius) transmitted geminivirus (WTGs), known as cotton leaf curl virus (CLCuV) (Hameed *et al.*, 1994). Viruses of this group belong to family Geminiviridae genus Begomovirus (Rybicki and Fauquet, 1998). Begomoviruses infect dicots and usually have two genome components (DNA-A and DNA-B) or of one DNA-like molecule, of circular single-stranded DNA. Until now no evidence of DNA-B have been found in CLCuV, however, several distinct variants of CLCuV-PK DNA has been reported (Zhou *et al.*, 1998). Characteristic symptoms of the disease include leaf curling, vein thickening, and sometime enation formation on the undersized of the leaf. Two types of leaf curling i.e. upward (UpW) and downward (DnW) are generally observed. Both types of symptom are manifested by all cotton cultivars in naturally infected as well as artificially inoculated cotton plants. Mixed type DnW and UpW symptoms can also be seen.

Most of the plant viruses are systemic in nature, that is, they spread from site of infection to other parts of the plant. Following infection, the virus begins to replicate in the host cell and alters cell's metabolism which results in biochemical and physiological changes. Symptoms are the observable effects that a virus has on the growth. A number of factors are known to influence symptoms caused by plant viruses. These include time of infection, plant age, virus strain, host's genetic makeup, environmental conditions and type of infection (mixed or single).

Materials and Methods

Sample collection: Samples were collected during cotton season 1996-97 from seven different locations viz. Central Cotton Research Institute (CCRI) Multan, Alabad, Neel Kot Bosan Road, Kohiwala, Cha Jahaz Wala Bosan Road, Manisal and Khokhran. Plants showing severely CLCuV symptoms were selected and two samples per field, one with UpW and other with DnW-curling were collected. A total number of 26 samples for determination of virus titer in UpW and DnW-curled and 34 for establishing the epitope profile were collected.

Epitope profile: Serological relationships among virions of different whitefly transmitted Geminiviruses (WTGs) are common, therefore, antisera (polyclonal and monoclonal) raised against one WTGs can be used for detection of heterologous WTGs (Roberts, *et al.*, 1984; Thomas *et al.*, 1986). Epitope profile of collected samples were established by using polyclonal to African cassava mosaic virus (ACMV) and monoclonal antibodies (MAbs) to ACMV (SCR 18, 20 & 23), Indian cassava mosaic virus (ICMV-SCR 52, 53, 55, 56, 58 & 60) and okra leaf curl virus (OLCV- SCR 106) by Triple Antibody Enzyme-Linked immunosorbent Assay (TAS-ELISA) as described by Harrison *et al.* (1997). Samples were loaded in duplicate in Nunc microtitre ELISA-plates.

Virus titer: TAS- ELISA was performed to measure CLCuV titer in UpW and DnW curled samples separately as described by Harrison *et al.*, (1997) by using MAbs SCR-60. The leaf tissues were crushed in extraction buffer (0.05 M Tris-HCl, pH 8.0, containing 0.06 M sodium sulphite (Tissue 1g : Buffer 10ml). Readings were taken at 405 nm after overnight incubation with substrate at 4°C.

Statistical analysis: To ascertain difference between UpW and DnW curling in relation to virus concentration and epitope profile of samples, T-test at 1 per cent level of significance was applied by using Statistical software MSTAT-C.

Table 1: Epitope profile of cotton leaf curl virus (CLCuV) infected samples with UpW and DnW symptoms.

S#	Type of symptoms	MAbs used									
		18	20	23	52	53	55	56	58	60	106
1.	Dn**	2*	2	0	2	2	1	2	2	2	0
2.	Dn	1	0	0	0	0	0	0	0	2	0
3.	Dn	3	T	0	2	0	2	t	0	3	0
4.	Dn	2	1	0	2	2	t	2	2	3	0
5.	Dn	2	T	0	3	0	1	0	0	3	0
6.	Dn	1	1	0	1	2	t	2	2	2	t
7.	Dn	T	0	0	1	0	0	0	0	2	0
8.	Dn	T	0	0	2	1	t	t	t	3	0
9.	Dn	0	0	0	0	0	0	0	0	t	0
10.	Dn	0	0	0	0	t	0	0	t	1	0
11.	Dn	0	0	0	2	0	t	0	0	3	0
12.	Dn	T	0	0	2	1	0	t	t	2	0
13.	Dn	4	0	0	4	0	3	2	3	4	0
14.	Dn	1	0	0	4	4	0	4	4	4	0
15.	Dn	0	1	0	t	1	0	0	t	1	0
16.	Up***	1	2	0	2	2	0	2	2	2	0
17.	Up	1	2	t	2	2	0	2	2	2	0
18.	Up	T	T	0	1	0	0	0	t	1	0
19.	Up	0	0	0	0	0	0	0	0	0	0
20.	Up	T	0	0	0	0	0	0	0	1	0
21.	Up	0	0	0	t	1	0	t	1	1	0
22.	Up	2	0	0	2	0	2	t	0	3	t
23.	Up	0	T	0	t	1	1	t	1	1	0
24.	Up	0	0	0	0	0	1	0	0	0	0
25.	Up	T	0	0	t-1	0	0	0	0	2	0
26.	Up	0	0	0	0	0	0	0	0	t	0
27.	Up	0	0	0	0	0	t	0	0	1	0
28.	Up	T	0	0	2	0	0	0	0	3	0
29.	Up	0	0	0	0	t	t	0	0	1	0
30.	Up	T	0	0	t	1	0	t	0	2	0
31.	Up	0	0	0	0	0	0	0	0	t	0
32.	Up	4	1	0	3	3	2	3	3	4	0
33.	Up	3	0	0	4	0	2	1	1	4	0
34.	Up	1	1	0	1	2	0	1	2	2	0

* Reaction strength
4 = strong, 3 = moderate, 2 = slight, 1 = weak, t = trace, 0 = none

** Dn= downward curling of leaves *** Up= upward curling of leaves

Results and Discussion

Statistical analysis of virus titer with type of symptoms (UpW or DnW) showed that virus titer is independent of symptoms type. Bar graph (Fig. 1) shows differences between virus titer with UpW or DnW curling in all places (Alabad, Neel Kot Bosan Road, Kohiwala, Cha Jahaz Wala Bosan Road, Manisal and Khokhran) except Alabad. Virus concentration in CCRI Multan, Cha Jahaz Wala Bosan Road and Manisal in case of downward curling is greater than upward curling. But the reverse is true for one location (Neel Kot Bosan Road) where it is higher than downward curling. Higher virus titer was visible in five locations in DnW curling, but opposite in one (UpW) and of equal amount at Alabad. However, statistically these values non significant, thus, no difference was found. Variation epitope profile of the tested samples does exist (Table but statistical analysis showed no relationship with typ curling (Table 2).

It is evident from this study that no difference ex between virus titer and epitope profile with type symptoms, thus no relationship could be establish Number of factors such as time of infection, variant/variants, environmental conditions, genoty combination of these or other unknown factors might T influence type of symptoms produced. In our view CL variants may play an important role on type of sympt

Table 2: Mean values of MAbs used for upward and downward curling.

MAbs used	Upward	Downward	t-values
18	0.8	1.1	0.574 ns
20	0.4	0.3	-0.269 ns
52	1.2	1.7	0.979 ns
53	0.8	0.9	0.870 ns
55	0.5	0.5	-0.209 ns
56	0.6	0.8	0.633 ns
58	0.8	0.9	0.880 ns
60	1.9	2.3.	0.344 ns

ns = non-significant at alpha = 0.01

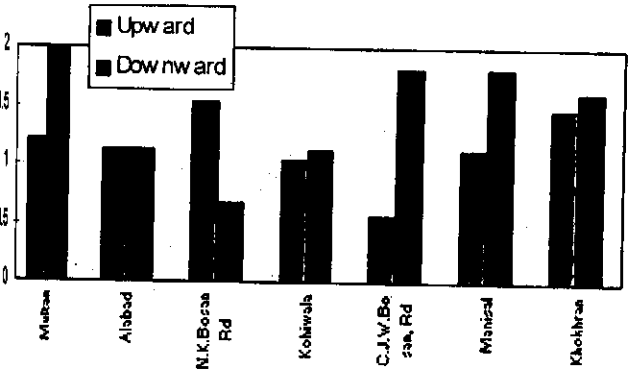

Fig. 1: Difference of virus concentration in relation to upward and downward curling.

Four CLCuV variants are reported to exists in the field (Zhou *et al.*, 1998), but we believe that more than four are prevalent in the field, which is evident from the epitope profile data (Table 1). Moreover, multiple infection (more than one variant of CLCuV and other WTGs) have been found to infect cotton and other crops in the cotton belt (data not shown). Therefore, it is worth investigating the effect of four known CLCuV variants, solely and in different combination, on type of symptoms. However, it should also be kept in mind that symptoms are influenced by other factors mentioned earlier.

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