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NATURAL OCCURRENCE OF TOBACCO STREAK VIRUS IN COTTON IN PAKISTAN AND SCREENING FOR ITS RESISTANT SOURCES

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Abstract

During 1994, a cotton mosaic disease was observed on the leaves of cotton plants at several locations in cotton growing areas of the Punjab. Infected leaves were showing typical symptoms of mosaic. Twenty six different cotton varieties belonging to the *Gossypium hirsutum* group were screened against cotton mosaic under the natural infection conditions in different ecological zones. Among these, cotton varieties CIM-70, S-12, B-622, B-30, B-496, BH-4, BH-89, BH-94, BH-95, and Krishma showed resistance to mosaic but these were highly susceptible to cotton leaf curl virus (CLCuV). None of the cotton varieties tested was resistant to both CLCuV and Tobacco Streak Virus (TSV). Based on ELISA, TSV was detected in samples showing mosaic symptoms. TSV was readily graft transmissible but not transmissible by mechanical means. No evidence of its transmission through seeds or by thrips was obtained.

Introduction

Cotton (*Gossypium hirsutum* L.) is the most important cash crop of Pakistan. It provides fiber and edible oil for human consumption and by-products of cotton seed serve as feed for dairy animals (Anon., 1990). Cotton plant is naturally susceptible to a number of diseases (Ahmed & Nelson, 1997) but those caused by viruses such as cotton leaf curl virus (CLCuV), cotton leaf crumple virus are most serious which can hamper the cotton production. Presently, CLCuV is a serious problem in cotton in Pakistan (Waqar, 1992). As a result, the cotton production has significantly decreased during the last decade. During 1994, while studying the epidemiology of CLCuV, cotton plants showing mosaic type symptoms on cotton were observed at several locations in major cotton growing areas of Punjab. This mosaic disease was not as serious as CLCuV and losses caused by mosaic were not significant. However, investigations on cotton mosaic disease were carried out with the idea that cotton breeders, who are actively engaged in evolving resistant varieties against CLCuV, may also consider this disease and adopt suitable strategies to tackle the problems of cotton leaf curl and cotton mosaic at the same time. A preliminary report regarding occurrence of TSV in cotton was published in the form of an abstract (Ahmed & Nelson, 1997).

Materials and Methods

Virus transmission: Transmission of cotton mosaic through seed was studied in pots by sowing 100 seeds of each 4 highly susceptible cotton varieties to mosaic disease i.e. CIM-1100, LRA-5166, CIM-434 and FH-634. The plants were kept in an insect free

glass house. Seeds used in this study were collected from severely naturally infected plants.

Insect transmission was attempted by collecting thrips from mosaic infected plants. Batches of 15-20 thrips were allowed to feed on plants of 4 highly susceptible cotton varieties at 2 leaf stages. After 48 hours of inoculations, plants were sprayed with an insecticide Roger (Dimethoate) @ 4 ml per liter of water and kept in an insect-free glasshouse.

Stem grafting was performed while taking infected plants (variety S-14) as root stock and healthy cotton plants (variety S-12) as scion. After grafting, plants were kept at 25°C ± 5°C in insect free greenhouse. Sap-inoculations were carried out on tobacco and cotton plants by grinding infected cotton tissue (dried or fresh) in 0.1 M phosphate buffer, pH 7.2. Prior to inoculations, plants were dusted with 600-mesh carborundum powder. The triturate was rubbed on the leaves of tested plants with a pad of cheesecloth. Inoculated plants were immediately washed with tap water. After 4-7 days, plants were assayed for local lesion assay.

Serology: Indirect Enzyme liked Immunosorbent Assay (indirect-ELISA) was performed as reported by Kaiser *et al,* (1991). The extract from infected tissue samples was diluted 1:10 in phosphate buffered saline (PBS), pH 7.4 with 0.05 % Tween-20, Ovalbumin (0.2%) and PolyVinylpyrrolidone (2 %) and adsorbed to polystyrene plates (Dynatech Laboratories, Inc. Alexandria, Virginia, USA) at room temperature for 2 hour. After adding immunoglobulin G (IgG) prepared against TSV in above mentioned buffer, the plates were incubated for 2 hour at room temperature. Goat anti-rabbit IgG, alkaline phosphate conjugate was diluted 1:1000 in the above buffer applied to the plates and incubated for 2 hours at room temperature. Plates were washed three times between each step with PBS-Tween 20. The substrate (p. Nitro phenyl phosphate) tablet was dissolved (1mg/1ml) in substrate buffer pH 9.8, added to the plates. The absorbance was recorded at A405 nm.

Screening for resistance: Seeds of cotton varieties used in this study were obtained from cotton breeders of the country belonging to Cotton Research Institute, Faisalabad; Cotton Research Station, Multan; University of Agriculture, Faisalabad; Nuclear Institute of Agriculture and Biology, Faisalabad and Central Cotton Research Institute, Multan. During 1997, 13 different cotton varieties were sown at the Punjab Seed Corporation (PSC) Farm, Khanewal in May 1997, 16 varieties at Cotton Botanist's Farm, Sahiwal in June 1997 and 13 varieties in the experimental area of Plant Virology Section, Ayub Agricultural Research Institute, Faisalabad in June 1997. In 1998, 15 cotton varieties were sown in the research area of Plant Virology, Ayub Agricultural Research Institute, Faisalabad and 12 varieties at PSC Farm, Khanewal during June 1998. Three rows of each variety were sown with plant to plant distance of 35cm and row to row distance of 75 cm. Observations on the severity of the cotton mosaic disease was recorded by using 0-4 scale follows:

0 = no symptoms
1 = 1-20 lesions on the leaf
2 = 21-50 lesions on a leaf
3 = 51-100 lesions on a leaf and
4 = more than 100 lesions on a leaf

Observations of CLCuV were recorded by using 0-6 scale which was evolved by the plant virologists of Pakistan and adopted for recording CLCuV data by all cotton breeders.

Results and Discussion

The symptoms of cotton mosaic recorded on different cotton varieties included formation of many small (1-4 mm diameter) yellow to light green spots which usually covered the entire leaf surface (Fig. 2). However, some plants produced comparatively bigger mosaic lesions which were yellowish in colour and their size ranged between 2-8 mm in diameter. Severely infected cotton plants were easily distinguished by their light green appearance in fields. It was noted during 1994 that under natural infection conditions, mosaic symptoms started appearing late in the season. Therefore, losses caused by the disease were not significant and disease was not in severe form. Similar results have been reported earlier by Costa (1995) who reported that in Brazil, cotton mosaic caused by TSV, appeared late in the season. This infection may be attributed to either through pollens or by thrips or both. However, our observations suggest that mosaic infection generally appears late in the season and is not consistent every year.

In this study, virus could not be transmitted either through sap inoculations or by thrips. ELISA results strongly suggested that cotton mosaic is caused by tobacco streak virus (TSV) belonging to the genus Ilarvirus, as all the infected leaf samples with variable symptoms, gave positive reaction against polyclonal antiserum of TSV in indirect ELISA.

Natural occurrence of cotton mosaic virus in the cotton belt of Punjab is shown in Fig. 1. It was noted that in 1998, mosaic symptoms started to appear 3-4 weeks after germination of plants i.e., much earlier of flower opening. In some varieties like S-14, 45 percent disease incidence was recorded in the 2nd week of July. This strongly supports that virus infection in cotton plants is carried out through insects (thrips) and not by pollens. Unusual high temperature and subsequently heavy rains in the month of June and July may also have played some role in the transmission of the disease. During the years from 1996 to 2000, mosaic infection in the field remained at its minimum level and found only in traces. However, in 2001, it again appeared in its severe form and was found on almost all the susceptible varieties. This suggested that comparatively low temperature and high humidity favours the disease development. In Pakistan from 1996-2000 there were very less rain compared to previous years thus climate remained dry and drought like conditions prevailed. But during the year 2001, rainfall was above normal and dry spell also ended. Moreover, before 2000 this disease could only be found in traces in the Multan region but in 2001 the mosaic disease appeared in severe form. This suggests that the disease is spreading year after year due to favourable climatic conditions. These results indicate that mosaic infection varies with the years and environmental conditions. In earlier studies, it has been reported that soil, climate, manuring, application of insecticides etc., can play an important role in the appearance of mosaic symptoms (Bink, 1975).

Results of screening trials showed that varieties CIM-1100, CIM-434, FH-634, BH-100, S-14, FH-633, FH-646, FS-628 and FH-679 are highly susceptible to cotton mosaic whereas the varieties FH-672, FH-645, CIM-240, FH-643, FH-679, Niab-92 and B-557 are moderately susceptible (Table 1, 2). Cotton varieties S-12, CIM-70, Krishma, B-94,

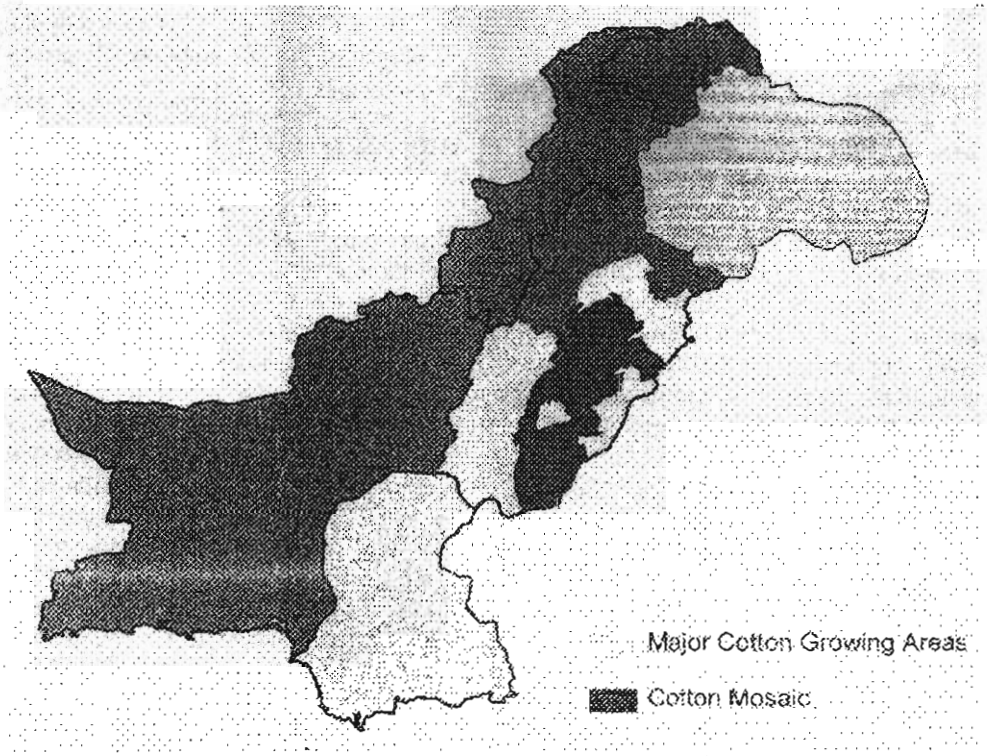

Fig. 1. Presence of cotton mosaic disease in Pakistan.

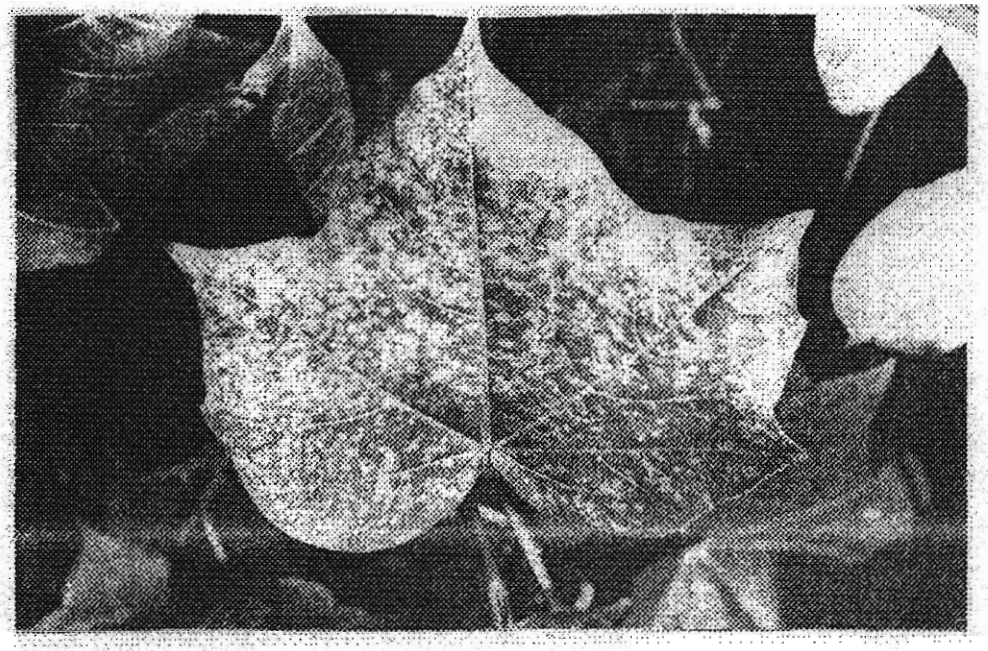

Fig. 2. Cotton leaf showing numerous light yellow mosaic lesions.

Table 1. Reaction of different cotton varieties against cotton mosaic disease caused by TSV under the natural conditions at Faisalabad, Khanewal and Sahiwal during the year 1994.

S. No.	Variety	Mosaic disease reaction on 0-4 scale		
		Faisalabad	Khanewal	Sahiwal
1.	FH-679	3	2	3
2.	FH-633	3	N.T.	N.T.
3.	FH-672	2	3	2
4.	FH-645	3	2	2
5.	FH-646	3	3	3
6.	FS-628	3	N.T.	N.T.
7.	CIM-1100	4	4	4
8.	CIM-240	2	3	3
9.	CIM-70	0	N.T.	N.T.
10.	LRA-5166	4	4	4
11.	S-12	0	0	0
12.	CIM-434	4	4	N.T.
13.	NIAB-92	N.T.	1	1
14.	FH-643	N.T.	2	1
15.	FH-634	N.T.	4	N.T.
16.	B-557	N.T.	N.T.	2
17.	BH-4	N.T.	N.T.	0
18.	BH-94	N.T.	N.T.	0
19.	BH-95	N.T.	N.T.	0
20.	BH-100	N.T.	N.T.	4

N.T.: Not tested.

Table 2. Reaction of different cotton varieties against cotton mosaic disease caused by TSV under the natural conditions at Faisalabad and Khanewal during the year 1995.

S. No.	Variety	Mosaic disease reaction 0-4 scale	
		Faisalabad	Khanewal
1.	S-12	0	0
2.	LRA-5166	4	4
3.	CIM-1100	4	4
4.	BH-100	4	4
5.	FS-628	3	4
6.	B-496	0	0
7.	B-622	0	0
8.	B-630	0	0
9.	FH-643	2	2
10.	FH-679	2	1
11.	CRIS-5/A	N.T.	3
12.	BH-89	N.T.	-
13.	BH-95	N.T.	-
14.	S-14	4	2
15.	KRISHMA	0	0

N.T.: Not tested.

Table 3. Reaction of different cotton varieties against cotton leaf curl virus and Tobacco Streak Virus under natural field conditions.

S. No	Variety	Disease Reaction against	
		Leaf Curl	Mosaic
1.	S-12	++++	-
2.	CIM-70	-	-
3.	CIM-434	-	++++
4.	FH-634	-	++++
5.	BH-100	-	++++
6.	CIM-1100	+	+++
7.	FH-633	+	+++
8.	FH-645	+++	++
9.	FH-646	+++	+++
10.	FS-628	++++	+++
11.	FH-672	++++	++
12.	FH-679	++++	+++
13.	S-14	++++	++++
14.	B-4	+++	-
15.	B-89	+++	-
16.	B-95	+++	-
17.	B-496	+++	-
18.	B-622	+++	-
19.	B-630	+++	-
20.	Krishma	+++	-

- = No symptoms, + = Tolerant, ++ = Moderately susceptible, +++ = Susceptible, ++++ = Highly susceptible.

B-95, B-622, B-630 and B-496 showed resistance against cotton mosaic but all these varieties are susceptible to CLCuV (Table 3). As reported by Ahmed & Nelson (1997), Bink (1975), Cauquil & Follin (1983), Nelson *et al*, (1998) and Nelson *et al*, (1998) this disease is considered to be a minor problem in Pakistan.

Other mosaic diseases like African mosaic (not caused by TSV) may also infect cotton plants, has been reported from Ghana, Chad, Mali, Nigeria, Tanzania, Togo etc. Cotton variety BJA 592 (*G. hirsutum*) was severely damaged in Chad from 1968 to 1970. In 1970-71, BJA-592 was replaced with tolerant variety Hg-9 followed by varieties Y-1422 and SRI-F4. Since then mosaic disease has virtually disappeared from Chad (Cauquil & Follin, 1983; Kraemer, 1966). However, variety BJA-592 which is susceptible to African mosaic is resistant to Central American mosaic. African mosaic and Central American Mosaics both are transmitted by white flies and infected cotton plants may become stunted. In some cases, due to the absence of flowers, total sterility is developed (Kraemer, 1966). Venial mosaic which produces typical chlorotic patches bounded with in the veins (Costa, 1960) and Mosaico which may produce crinkled and deformed limbs (Tarr, 1964) has also been reported from cotton. The causal agents of all these mosaic viruses are not known. In our study, all the diseased samples frequently tested at different plant stages by indirect-ELISA, showed positive reaction against TSV antibodies which strongly suggested that mosaic disease found in Pakistan is caused by TSV. Initial studies showed that virus is not transmissible through sap-inoculation from

cotton to cotton and cotton to tobacco plants. In graft transmission studies, typical mosaic symptom started to appear on leaves after 4-5 weeks of grafting. Disease symptoms were not produced in any plant in all 4 tested varieties grown for seed transmission studies. When ELISA was perfórmed on these plants, antigen could not be detected in any tested plant except from positive controls. After growing for 12 weeks, tested plants were discarded.

No insect vector has been identified for cotton mosaic in Pakistan as symptoms could not be developed on plants inoculated with thrips. For that reason, the vector of the disease remained unknown. Virus also could not be detected from these sap-inoculated plants. However, TSV is reported to be transmitted by thrips (Kaiser *et al.*, 1982) and may infect many plant species (Fulton, 1985). The most important reservoir hosts of the virus are white sweet clover and alfalfa (Hampton, 1967; Paliwal, 1982). Other important hosts of TSV are Chickpea and beans (Kaiser *et al.*, 1991; Kaiser *et al.*, 1998). All these hosts are present in the cotton areas where cotton mosaic is found. Therefore, host-virus-vector interaction needs to be studied in detail.

Most of the symptoms induced by the infection of cotton mosaic are similar as reported earlier by Cauquil & Follin (1983). In this study, stunting and sterility of plants did not occur both in early and late infection under the field and greenhouse studies. It is interesting to note that cotton varieties S-12, CIM-70 etc., which are highly susceptible to CLCuV are highly resistant to cotton mosaic while cotton varieties CIM-434, FH-634 and BH-100 which are highly resistant (on the basis of symptoms) to CLCuV are highly susceptible to cotton mosaic. This indicates that plants of these varieties carry different genes which may confer resistance against both the viruses. It is however, believed that some cotton varieties are susceptible to both CLCuV and TSV infection (Table-3). However, no synergistic effect occurs in plants which are infected simultaneously with CLCuV and cotton mosaic virus. No cotton variety included in the study has been found which possess resistant genes for both CLCuV and cotton mosaic viruses.

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