Cotton Leaf Curl Virus: A Threat to Pakistan Cotton

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ABSTRACT

Spread of cotton leaf curl virus (CLCuV) in cotton growing areas of Punjab cause immense losses in cotton production in Pakistan. It appeared in epidemic form in 1998, but became serious from 1992-93. Studies to combat this problem centres around the characterization of virus, its vector (whitefly), alternate host and the causative environmental factors. In our surveys, it was found that the incidence and severity of the disease varied from place to place, developing hot spots under certain environmental conditions that we tried to analyze. The nature of this virus was studied through molecular characterization. Studies were undertaken to develop cultivars resistant to the virus. A technique for the field evaluation of the breeding material on a large scale was developed which helped to screen for resistance to the disease. This helped to show that this disease is controlled by a single dominant gene. A series of resistant cotton strains have been developed since then, that save the crop from the onslaught of the virus. Two resistant cultivars, CIM – 1100 and CIM – 448, have already been approved for general cultivation. Future behaviour of the virus is being monitored.

Introduction

Cotton plays a key role in the economy of Pakistan. It contributes about 60% of the total foreign exchange earnings through the export of raw cotton and cotton products. It also provides raw material to local domestic cotton industry comprising over 503 textile mills, 1135 ginning factories and over 5000 oil expelling units. It has an 85% share in the total vegetable oil produced in the country. Cotton seed cake, an important by-product, is a valuable source of protein for ruminant cattle. 40% of the country's labour force is employed in cotton fields and cotton processing mills. Due to its multifarious benefits cotton is rightly described as the backbone of Pakistan's economy.

Pakistan ranks fourth in area and production of cotton in the world. It has 9.36% of total world cotton area, 10.18% of production, 8.06% of consumption and 4.55% of total world export of raw cotton.

Area, Production and Yield

Since independence in 1947, the area, production and yield have showed a rising trend. Increase in production is due more to increase in yield/ha rather than increase in area. However, during some years the production and yield/ha have shown considerable fluctuations, attributable to bad weather conditions, especially high rainfall, floods and heavy onslaught of insect pests and diseases (Figure 1).

The highest ever production of 12.8 million bales, was achieved in 1991-92 but there was a downward trend in the subsequent two years and production dropped to 8.04 million bales in 1993-94 due to a severe out-break of cotton leaf curl disease (CLCuV) which caused heavy damage to the cotton crop. Since then yield loss has been a regular occurrence every year due to CLCuV. About 6.5 million bales have been lost during the last decade (Table 1).

However, as a result of the increase in area under relatively tolerant/resistant cotton cultivars and other remedial measures against CLCuV the declining trend in cotton production has not only been checked but upward and the country produced 10.06 million bales in the year 1995-96. During 1996-97, however, production again declined to 9.35 million bales as a result of severe attack of whitefly, leaf curl virus and continuous hot and dry weather.

History of the disease

Cotton leaf curl disease was first reported from Multan in 1967. It was considered a minor disease until 1987 (Hussain and Mahmood, 1988). In 1988, it damaged the cotton crop on 60 hectares in Multan district. Since then it has been increasing every year. It affected 200 hectares in 1989 and 800 hectares in 1990. In 1991, the disease appeared in epidemic proportions over an area of 14,000 hectares in the Multan, Khanewal and Vehari districts. In 1992, it spread to more than 485,000 hectares, causing a decrease in production and significant monetary loss to the country. In 1993, the disease spread to the entire Cotton Belt of the Punjab with varying intensity and damage to the crop on 889,000 hectares. The disease was also reported from D.G. Khan and Sindh.

In Pakistan, studies on transmission and incidence of CLCuV were first undertaken at the Central Cotton Research Institute (CCRI), Multan in the year 1974-75. The disease was noted on a number of plants of different cultivars 149-F, AC-134, Deltapine, Dunn,

Proceedings of the World Cotton Research Conference-2. Athens, Greece, September 6-12, 1998. pp.936-940.

Acala and Carolina Queen in the Multan area. Similar symptoms were also noted on *Althea rosa, Hibiscus rosa-sinensis, Hibiscus esculentus* and *Poinsettia* sp. Transmission of the disease from cotton to cotton has been achieved through grafting. Insect transmission using whitefly from cotton to cotton and okra to cotton was quite successful. Transmission from seed, soil or mechanical contact gave negative results.

Regular studies on the disease were started at the Institute after the first epidemic in 1988. The work was primarily oriented towards the screening of the germplasm and development of CLCuV resistant cultivars. Other topics were added later in the course of time.

Mohsin *et al.* (1992) isolated virus from CLCuV affected leaves. After examination under electron microscope, it was concluded the virus belonged to Gemini group that has a whitefly as the vector. The transmission was reported by Markham, (1992). Hashmi *et al.*, (1993) confirmed that CLCuV was a Geminivirus and proved that a whitefly is the vector of CLCuV. They also claimed successful purification and identification through electron microscopy of CLCuV as Gemini virus. In 1993, Mansoor (NIBGE, Faisalabad) confirmed the association of geminivirus with leaf curl disease by OCR and sequence analysis. This was the first ever report published about the characterization of the virus. While studying the molecular characteristics of cotton leaf crumple virus (originating in North America) and CLCuV (originating from Pakistan), Nadeem *et al.* (1994) concluded that these viruses are only distantly related. Mirza (1992) attempted serological identification of the virus. Tests were carried out using monoclonal antibodies prepared from Indian Cassava mosaic virus through TAS ELISA. The results of the serological tests confirmed the presence of a geminivirus on infected cotton. Khalid (1994) reported that CLCuV particles were observed by immunosorbent electron microscopy and also detected the virus by PCR in plant tissue and in single virus-carrying *B. tabaci*. The viral DNA encoding the virus particle protein was cloned onto a plasmid, and will be used to prepare a non-radioactive probe for CLCuV.

Breeding Resistant Cultivars

The first step was to find existing cultivars (*Gossypium hirsutum*) that could withstand an attack of the virus. None of the cultivars was found to be resistant but there were different grades of tolerance in various cultivars. Besides testing the existing cultivars, efforts were made to evolve new strains with resistance to CLCuV. As a first step the genetic stock involved in the hybrids was carefully studied and it was discovered that CP-15/2 and LRA-5166 maintained resistance to CLCuV. Stress was laid on observations in hybrids having these two cultivars as parents. The progenies 1098 and 1100 out of the cross 492/87 x CP-15/2 in the crop season 1992-93 emerged as the first lines resistant to CLCuV. Line 1100 was identified for detailed analysis. Crosses between local cultivars and exotic virus resistant cultivars attempted in 1988 at the Central Cotton Research Institute, Multan lead to the development of two virus resistant cultivars, CIM-1100 and CIM-448 that were approved by the Punjab Seed Council in 1996 for general cultivation in the Punjab. Besides possessing good yield potential, these two cultivars have 38% ginning out turn and 29 mm (1-1/8") staple length. They increased to about 20.1% area in the Punjab during 1997-98 (Table 2). In addition to this a number of other virus resistant cultivars are in the pipeline. A virus resistant cultivar suited to Faisalabad conditions, FH-634, was developed at Ayub Agricultural Research Institute, Faisalabad.

A new technique consisting of creating high virus inoculum pressure for screening plants for susceptibility to virus, was developed. A "sick bed" was designed where virus disease pressure would be at its maximum along with the environmental conditions conducive to the development of virus and the development of symptoms on the plant. Ready inoculum was made available in the form of ratooned plants of a susceptible cultivar that would keep inoculum available from the date of sprouting (mid February) onwards till end of crop season. Whitefly, the vector, required for transferring the virus from this source of inoculum to the test seedlings, is normally available throughout the year. Its population, however, was increased in spring season by planting berseem in a nearby field. The test material was then planted in between the ratooned lines where it would get exposed to disease pressure and seedlings could be identified for susceptibility or resistance to this disease with confidence.

Future Research

The impact of CLCuV on the Pakistan national economy cannot be forgotten as the country has lost Rs. 50 to 55 billions since 1992 and it is essential to maintain vigilance over the disease. Although some resistant cotton strains are in sight, this is just a beginning. These are yet to be tested for their agronomic traits including yield, technological properties and adaptability to the environment in various ecological zones. Dependence on these strains alone will not be enough. Genes in the virus and in the cultivars can mutate. Hybrids involving virus resistant parents from other countries, especially from Africa, also require study and exploitation. If more than one gene for resistance is discovered, the goal should be to develop multigenic resistance to this disease. The existing genetic stock from various countries should be augmented with fresh accessions for this purpose.

Different wild and cultivated species and some interspecific hybrids developed at CCRI, Multan, can provide valuable genetic variability for this purpose. A few virus resistant lines have already been isolated from crosses involving *G. arboreum* and *G. anomalum* species. Derivatives from this material can be crossed with *G. hirsutum* cultivars to incorporate the source of resistance. All this material should be subjected to biochemical tests through PCR or similar techniques and the plants showing resistance to CLCuV should be used for producing resistant transgenic plants by using the new techniques of genetic engineering.

The prospects for recovering from the vicious circle of cotton leaf curl virus infection are very bright. It is very encouraging that our cotton breeders have developed virus resistant cultivars in the shortest possible time. In other countries such as Nigeria and Sudan where the disease appeared in the 3rd decade of this century, they were unable to develop resistant cultivars for twenty years and they had to abandon cotton cultivation to get rid of the disease. In addition to the development and release of virus resistant cultivars, almost all cotton breeders in the Punjab have developed virus resistant breeding material that will ultimately lead to the development of virus resistant cultivars in the future. It is the policy of the government that in future no virus susceptible/tolerant cultivar should be approved for general cultivation. Under these circumstances, it is earnestly hoped that within the next two or three years the entire cotton area in the Punjab will be under virus resistant cultivars and the country will get rid of this menace.

Control Strategy

Based on the research information, Central Cotton Research Institute, Multan recommended the following management strategies for the control of this disease.

1. Resistant cultivars should be planted.
2. It is always advisable to plant more than one cultivar so as to create genetic barrier.
3. The previous year's cotton stubs should be removed from the fields because sprouts from diseased plant stubs transmit this disease.
4. Plants are susceptible in their early growth period. As soon as whitefly populations and disease symptoms start appearing, the spray regime based on the economic threshold level should be implemented for the control of whitefly.
5. As soon as the crop passes the seedling stage, judicious use of fertilizer and irrigation is recommended.
6. The crop should be protected from other sucking pests.
7. Normal practices of fertilizer, irrigation and plant protection should be followed.
8. Weeds and alternate host plants in and around cotton fields should be removed.
9. Inter-cropping of cotton in orchards should be avoided.
10. If attacked early in the season, tolerant cultivars have the capacity to recover from cotton leaf curl virus effects. The farmers are advised not to plough such fields but give normal treatments to crop that will recover, giving reasonable yields of seed cotton. This has been observed during recent crop seasons.
11. Monitoring of whitefly at regular intervals should be carried out with yellow sticky traps and as soon as populations reach the threshold level spray programme should be initiated.
12. Spraying is recommended in the early morning before while the whitefly is at its least active.

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Table 1. Losses due to cotton leaf curl virus 1988-99.

Year	Affected Area (000 ha)			Loss in Production
	Partial	Complete	Total	(000 bales)
1988-89	-	0.06	0.06	0.3
1989-90	-	0.20	0.20	1.0
1990-91	-	0.80	0.80	4.0
1991-92	11.3	2.80	14.10	20.0
1992-93	364.0	121.00	485.00	750.0
1993-94	607.0	282.00	889.00	1880.0
1994-95	407.0	-	407.00	221.0
1995-96	882.0	-	882.00	447.0
1996-97	1623.9	137.40	1761.30	2100.0
1997-98	762.9	19.50	782.40	1118.1

Table 2. Change in percent area of different cultivars in Punjab.

Cultivar	1991	1992	1993	1994	1995	1996	1997
MNH-93	28.17	23.95	29.63	24.07	9.73	6.15	3.6
NIAB-78	32.38	31.69	35.33	30.37	19.62	11.48	11.1
CIM-70	3.66	3.03	1.13	1.28	0.62	0.33	-
CIM-109	-	0.71	3.52	4.43	4.32	3.78	1.6
CIM-240	-	-	4.70	24.59	53.14	62.89	24.9
CIM-1100	-	-	-	-	-	0.82	16.0
CIM-448	-	-	-	-	-	-	4.1
S-12	33.29	39.26	22.82	10.46	6.88	6.86	0.9
SLS-1	-	-	-	-	2.22	3.80	3.4
BH-36	-	-	-	1.51	1.29	1.53	1.7
Karishma	-	-	-	-	-	0.29	17.1
Others	2.50	1.36	2.87	3.29	2.18	2.07	15.6

Source: Crop Reporting Service, Government of the Punjab, Pakistan.

Figure 1. Area, yield and production of cotton in Pakistan (1947-97).