Status of cotton leaf curl virus disease in India

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Introduction

Cotton leaf curl virus disease (CLCuD) earlier known as African leaf curl of cotton was reported for the first time from Nigeria on *Gossypium peruvianum and G. vitifolia* in 1912 by Faquharson. The disease was first reported in India on *G barbadense* at Indian Agricultural Research Institute, New Delhi in 1989. Subsequently it appeared in patches during 1993 around Sriganganagar district of Rajasthan and Ferozepur district of Punjab adjoining to Pakistan border on *G. hirsutum* and spread to entire north Indian cotton zone of around 15 lakh hectares in a short span of 4-5 years. CLCuD is caused by a complex of whitefly (*Bemisia tabaci*) transmitted Begomoviruses *having mo*nopartite genome with circular ssDNA associated with satellite (beta and alpha satellite) DNA molecules. The disease generally appears in the end of June about 45-55 days after sowing and spreads rapidly in July. The disease progress becomes slow in August and almost comes to a halt by mid October. The initiation of disease is characterized by small vein thickening (SVT) type symptoms on young upper leaves of plants. Upward/downward leaf curling and leaf thickening followed by formation of cup shaped leaf laminar outgrowth of veinal tissue (leafy enations) on the abaxial side of the leaves is another important symptom. An incubation period ranging from 10-18 days has been reported.

Background information

Research efforts made by state agricultural universities and ICAR institutions led to identification of resistant sources, development of resistant *G hirsutum* varieties / hybrids, development of molecular diagnostic tools, estimation of crop losses, epidemiological studies including development of disease maps, prediction equations and identification of weeds and other hosts (Monga et al., 2011)

The changing scenario and present status

The development of recombinants:

Studies from India and Pakistan have demonstrated a complex etiology of CLCuD involving CLCuV and a minimum of four other distinct and closely related, begomovirus species namely: Cotton leaf curl Alabad virus (CLCuAV), Cotton leaf curl Kokhran virus (CLCuKV), Cotton leaf curl Multan virus (CLCuMV), and Cotton leaf curl Rajasthan virus CLCuRV (Nadecm et al. 1997; Briddon and Markham 2000).Following resistance breaking in cotton in 2001, Cotton leaf curl Burewala virus (CLCuBuV) was found across Punjab, Pakistan (Amrao et al., 2010). In addition Cotton leaf curl Bangalore virus (CLCuBV), Papaya leaf curl virus (PaLCuV) and Tomato leaf curl Bungalore virus (ToLCuBV) are also associated with the disease (Geering 2010). Furthermore, each virus species has several strains and variants identified on the basis of disease prevalence in specific geographic areas(Fauquet et al. 2008: Kumar et al. 2010). In Africa the disease is caused by Cotton leaf curl Gezira virus (CLCuGV) that has great diversity in their sequences from the Asian viruses that cause CLCuD (Briddon 2003; Geering 2010). Recently, during a survey of symptomatic cotton in Sindh (southern Pakistan), Cotton leaf curl Gezira virus (CLCuGeV), the begomovirus associated with CLCuD in Africa, and a member of a new tentative begomovirus species referred to as *Cotton leaf curl Shahdadpur virus* (CLCuShV) were identified (Amrao et al., 2010; Tahir et al., 2011). Both CLCuBuV and CLCuShV have recombinant genomes, with sequences derived from CLCuKoV and CLCuMuV.

Until 2004, CLCuRV (Rajasthan strain) was predominant in north west India but during 2005-06, CLCuBuV (Burewala strain) appeared to be a new Begomovirus and in the recent years of 2009-10 onwards CLCuBuV emerged as dominant resistance breaking strain in NW India. A survey for viruses causing CLCuD was conducted by Rajgopalan et al (2012) during the 2009 and 2010 cropping seasons in the northwestern Indian cotton-growing belt in the states of Punjab, Haryana and Rajasthan. Partial sequences of 258 and full-length sequences of 22 virus genomes were determined. This study showed that the resistance-breaking cotton leaf curl Burewala virus (CLCuBuV) is now the dominant virus in many fields.

Six strains of CLCuV, including Sri Ganganagar strain isolated from a severely infected CLCuV-resistant variety, were characterized and nucleotide sequences of DNA-A and β DNA components were determined. Sequence comparisons revealed 81–99% and 88.3–92% sequence identity of DNA-A and β DNA, respectively, with known CLCuV sequences. Recombination

analysis revealed significant recombination in these six virulent Indian strains showing 25 recombination sites in DNA-A and 11 recombination sites in βDNA. The observed recombination in several regions of DNA-A and βDNA in the potential resistance breaking Sri Ganganagar strain of CLCuV was mapped to the highly virulent Burewala strain and several other strains (Chakrabarty et al,2011).

Although. CLCuV is a distinct virus, it shows considerable identity with other disease causing begomoviruses on other plants such as tomato. okra and some weed spp. The epitope profiles of CLCuV-PK (CLCuMV) were indistinguishable from the profiles of viruses causing yellow vein disease of okra in India or Pakistan or leaf curl of okra (*Abelmoschus esculentus*), Hibiscus tiliaceus, radish or sunflower in Pakistan (Harrison et al. 1997). The virus causing *Hibiscus* leaf curl disease (HLCuD) from Southern India and Pakistan showed 95-97% DNA-A nucleotide identity with CLCuMV (Rajeshwari et al. 2005; Mao et al. 2008). The virus of Yellow vein disease of *Digera arvensis* showed 98% nucleotide Sequence identity with CLCuRV (Mubin et al. 2009). Similarly, Tomato leaf curl virus (ToLCV) in Pakistan showed 99% sequence identity with CLCuRV (Shahid et al. 2007, 2009).

Resistance breakdown, yield loss trials and new germplasm screening initiative:

Based on a study conducted during 2013-14 season at three locations in north zone in India, it was observed that earlier known resistant varieties released from state agricultural universities of north zone and the tolerant Bt cotton hybrids were now showing susceptible to highly susceptible reaction (Table 1). All the cultivars which were resistant/tolerant to CLCuD, now show susceptible reaction probably due to appearance of recombinants and predominance of Burewala strain.

The yield loss assessment due to cotton leaf curl virus diseases at different locations of north India (Faridkot, Abohar, Ludhiana, Hisar and Sriganganagar) were carried out (2009-14) on fifteen Bt cotton hybrids popularly grown by the farmers (Table 2).Seed cotton yield reduction ranging from 15.7% to 56.7% was recorded (AICCIP Annual Report, 2012-13 & AICCIP Annual Report, 2013-14; Monga,2014).

Out of around 5000 germplasm lines screened against disease during 2011-14 at the regional station, not a single line free from disease has been observed. However, few lines showing

tolerance against the disease have been identified and used for making crosses with highly susceptible lines to make mapping population for marker assisted selection

Management options :

The available management options being advocated to the farmers are listed below:

- Ban on sale of non descriptive Bt hybrids and other genotypes.
- As *G. arboreum* are immune to CLCuD and high yielding desi cotton varieties/hybrids from public sector are available and give remunerative price also, cultivation of more such genotypes should be promoted in hot spot areas.This may be followed by cultivation of higher yielding and CLCuD tolerant Bt cotton hybrids.
- Avoid American (*G.hirsutum*) cotton cultivation in citrus orchards.Avoid cultivation of okra and other malvaceous hosts in hot spot areas.
- Clean cultivation drive after wheat harvesting. Remove weeds from road side and canals, citrus orchards and around cotton fields.
- Control vector whitefly at early growth stages of cotton with the help of neem oil @ 1liter/acre. Yellow sticky traps should be encouraged for reduction of white fly population.

Ten different interventions(T1- Whey protein @ 5%, T2- Cow urine 6.6%, T3- Neem oil @1%, T4- Mustard oil @ 3%, T5- Kaolin @ 2%, T6- Calcium nitrate @ 0.5%, T7- Potassium nitrate @ 0.5%, T8- Paraffin liquid @ 2% T9- Strobilurin @ 0.1%, T10- Acephate @ 0.4%) along with Control were tested in an randomized block design for two years (2011-13) at the station to see their efficacy in containing cotton leaf curl virus disease. Cow urine followed by kresoxim methyl and calcium nitrate showed percent disease incidence reduction from 52.85 in control to 34.15,34.20 and 37.40 respectively. PDI reduction from 20.4 to 10.6,11.2 and 13.3 respectively was also noted in these treatments (Monga,2014 personal communication, Table 3).

The future perspective

It is envisaged to study nature of disease resistance to strengthen development of resistant material and Introgression of resistance from wild species and diploid cottons, breed cotton leaf curl disease resistant varieties based on marker assisted selection and integrated vector management in context of CLCuD control in the coming years.

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Table 1: Monitoring of breakdown of resistance against CLCuD in cotton

Variety/ hybrid earlier reaction	**CLCuD Percent disease index (PDI)**				
Susceptible	**BHT**	**HSR**	**SNG**	**Cumulative mean**	**Cumulative reaction**
HS-6	61.4	50.6	76.7	62.9*	HS
RST 9	84.1	50.1	74.5	69.6	HS
F 846	54.5	49.0	67.8	57.1	HS
RCH 134	73.3	56.8	81.6	70.6	HS
Tolerant					
H-1236	45.5	46.7	74.4	55.5	HS
RS 2013	38.6	46.2	64.8	49.9	S
LH 2076	27.7	46.7	67.2	47.2	S
RCH 650	22.7	40.6	65.3	42.9	S
CD @ 5%	5.2	3.5	4.4		
CV %	7.1	4.1	4.3		

***As per rating scale: PDI- 40.1to 50-Susceptible; >50- Highly susceptible**

Table 2:Seed cotton yield reduction(%) due to CLCuD in different Bt cotton hybrids in north zone during 2009-14

2009-10		2010-11		2011-12		2012-13		2013-14	
hybrid	**% reduction**	**hybrid**	**% reduction**	**hybrid**	**% reduction**	**hybrid**	**% reduction**	**hybrid**	**% reduction**
RCH 134 Bt	45.4	RCH 134 Bt	42.9	MRC 7031 BG II	25.2	Ankur BG II	55.7	RCH 134 Bt	39.0
MRC 6304 BG	41.4			SP 7007 Bt	46.6	NCS 885 BG II	46.4	Ankur Jai Bt	38.1
				Jai BG II	43.1	RCH 134 Bt	56.7	NCS 855 BG II	33.3
				MRC 7017 BG II	27.0	RCH 134 BG II	49.9	RCH 134 BG II	46.3
				NS 858 BG II	30.4			Bioseed 6588	15.7
				RCH 134 Bt	40.8			Bioseed 6488	20.8
								Ankur 3028	18.0

Table 3: Effect of different intervention on incidence and severity of CLCuD (2011-13)

Treatment	% incidence	Percent disease index(PDI)	Seed cotton yield Kg/ha
T1= Whey protein@5%	40.25	14.3	889.6
T2=Cow Urine @ 6.6%	34.15	10.6	979.8
T3=Neem oil@ 1%	41.75	13.1	861.6
T4 =Mustard oil @ 3%	43.10	14.8	791.3
T5= Kaolin @2%	44.45	15.8	900.6
T6= Calcium nitrate @ 0.5%	37.40	13.3	866.3
T7 = Potassium nitrate@ 0.5%	49.10	20.1	912.1
T8= Paraffin Liquid@ 2%	45.10	16.6	979.4
T9= Kresoxim methyl@ 0.1%	34.20	11.2	922.1
T10= Acephate @ 0.4%	47.60	15.5	855.4
T11= Control	52.85	20.4	823.6
CD at 5%	7.16	2.85	
CV%	11.63	13.3	