Cotton leaf curl virus disease in India-Recent status and management strategies

D Monga, P K Chakrabarty and K R Kranthi
Central Institute for Cotton Research (C.I.C.R.), Regional Station, Sirsa, Haryana and C.I.C.R., Nagpur

Abstract

Cotton leaf curl virus disease (CLCuD), caused by a single stranded circular Gemini virus consisting of DNA-A and two satellites ie DNA-1 and DNA beta and transmitted by white fly (*Bemisia tabaci*) is an important problem of northern cotton growing region of India with area ranging from 12-20 lakh hectares in the states of Haryana, Punjab and Rajasthan during the last one and half decade. The initiation of disease is characterized by small vein thickening (SVT) type symptoms on young upper leaves of plants. Upward/downward leaf curling followed by formation of cup shaped leaf laminar out growth of veinal tissue on the abaxial side of the leaves are other important symptoms. In severe cases reduction of internodal length leading to stunting and reduced flowering/fruiting is also noted. The effective management of this important disease is possible by development of resistant varieties, suppression of whitefly and eradication of weed hosts carrying this disease. The disease after its report in patches around Sriganganagar district of Rajasthan on *G. hirsutum* in 1993 spread to entire north India in a short span of 4-5 years. It was noted that the most commonly grown cotton varieties in the northern states at that time ie RST-9 in Rajasthan, F-846 in Punjab and HS-6 in Haryana were severely hit as they were highly susceptible to this disease. Since the development of resistant variety /hybrid is the only reliable and cheaper method of its management, several resistant/tolerant varieties like RS-875, RS-810, RS-2013, F1861, LH-2076, H1117, H-1226, H-1236 and Hybrids like LHH144, CSHH198, CSHH238 and CSHH 243 were developed over the years by the SAUs and ICAR institutions working in the region. The disease was brought under control and the damage caused by it was considerably reduced. The Bt cotton hybrids were introduced in north India during 2005 as a strategy to combat the menace of bollworm complex. The major area(more than 90%) has now come under Bt cotton hybrids. Cotton leaf curl virus disease appeared in a severe form during 2009-10 crop season in some areas of north zone. The hitherto known resistant varieties also showed susceptible reaction at hot spot areas. Accumulation of recombination events over the years coupled with favorable environmental conditions appeared to have knocked down the resistance of cotton during 2009-10 season. The incidence of disease is increasing and this has become a threat to cotton cultivation in the region. Research efforts to develop resistant varieties/hybrids through conventional/biotechnological approaches along with cultural and vector management practices are in progress for effectively containing this disease.

Introduction

Cotton is the most important kharif cash crop of north India. Among the various factors responsible for its low production and productivity during the last one and a half decade, cotton leaf curl virus disease(CLCuD) has been found to be one of the major limiting factor. The disease has assumed serious proportions in the most potential irrigated cotton belt of north India comprising an area of around fifteen lakh hectares. The disease caused by a whitefly transmitted Gemini virus was first noticed in Nigeria on *Gossypium peruvianum* and *G. vitifolia* (Farquharson, 1912). In India, cotton leaf curl virus disease was first reported on American cotton (*G hirsutum*) in Sriganganagar area of Rajasthan state during 1993 (Ajmera,1994) and during 1994 it appeared in Haryana and Punjab (Rishi and Chauhan,1994;Singh et al,1994) states on *hirsutum* cotton and posed a major threat to its cultivation in northern India (Verma et al.,1995). The disease has appeared in an epidemic form during 1997 in the Rajasthan affecting an area of 0.1 million hactares (Anonymous,1998). The major area (more than 90%) has now come under Bt cotton hybrids. Cotton leaf curl virus disease appeared in a severe form during 2009-10 crop season in some areas of north zone. The hitherto known resistant varieties also showed susceptible reaction at hot spot areas. Recent advances made in development of new resistant varieties/hybrids, epidemiological studies including development of disease maps and detection of new weed hosts and breakdown of resistance due to development of new viral recombinants are discussed along with future management strategies.

Resistant varieties development program

A vigorous exercise was taken up by the state agricultural universities and ICAR institutions in the region to work out strategies for its management. The disease could be managed by development of resistant

varieties/hybrids, control of its vector whitefly and eradication of weeds harbouring cotton leaf curl virus disease (Narula *et al*, 1999; Monga *et al*, 2001). Molecular diagnostic tools for detection of virus were developed.(Chakrabarty *et al*, 2005) The most commonly grown varieties in the northern states affected by this disease ie RST-9 in Rajasthan, F-846 in Punjab and HS-6 in Haryana were highly susceptible. Since the development of resistant variety /hybrid is the only reliable and cheaper method of its management (Narula et al., 1999) several resistant/tolerant varieties(Table-1) like RS-875, RS-810, RS-2013, F1861, LH-2076, H1117, H-1226 and Hybrids like LHH144, CSHH198, CSHH238 and CSHH 243 were developed over the years by the SAUs and ICAR institutions working in the region (Ajmera et al., 2004, Radhakrishnan et al., 2004, Anonymous 2005 and 2006, Tuteja et al, 2005, Tuteja et al,2006, Tuteja et al,2009). The disease was brought under control and the damage caused by it was considerably reduced. The Bt cotton hybrids were introduced in north zone during 2005 as a strategy to combat the menace of bollworm complex. Initially six hybrids approved by Genetic Engineering Approval Committee were permitted for cultivation in north zone. Subsequently, however, a large number of hybrids were permitted for cultivation with in a span of five years and amongst them a number of hybrids were observed to be susceptible to cotton leaf curl virus disease. At present, high yielding CLCuD tolerant Bt hybrids screened under field conditions are identified and advocated to the farmers.

Table 1. Promising CLCuV resistant varieties/hybrids released in India (north zone)

Name of Variety/hybrid	Source
H-1117, H-1226, H-1236	Chaudhary Charan Singh Haryana Agricultural University, Hisar,Haryana
F-1861, LH-2076	Punjab Agricultural University, Ludhiana,Punjab
RS-875, RS-810, RS-2013	Rajasthan Agricultural University, Sriganganagar, Rajasthan
LHH-144 (Hybrid)	Punjab Agricultural University, Ludhiana,Punjab
CSSH 198,CSHH-238,CSHH-243 (Hybrids)	Central Institute for Cotton Research,Regional Station, Sirsa, Haryana

Disease epidemiology

Occurrence and prediction of cotton leaf curl virus disease

Disease incidence, its progress, whitefly population and weather parameters were recorded from 27th to 37th week continuously for eleven years under screening nursery and simple correlations and multiple regression analysis were worked out to study the influence of weather factors on whitefly population and progress of disease. During 37th week, maximum disease incidence (98.0%) was observed during 2001 followed by 2000 (95.4%) and 2006 (93.8%) where as it was minimum during 2008 (2.7%) followed by 2004 (18.5%) and 2007 (35.4%)(Table-2). Minimum whitefly population of 0.05 (per 3 leaves) was noted in 27th week during 2002 and 2008 and maximum of 2.3 was observed in 37th week during 2009(Table-3). Multiple regression analysis was worked out to identify the factors helpful in progress of disease during 27 to 31, 32 to 37 and 27 to 37 weeks. Best fitted multiple regression equation of 27th to 31st standard week was: $Y_{10} = 199.507 – 6.266X2_{120} – 3.335X_{121} + 0.683X_{133} + 0.552X_{140} + 0.409X_{144} – 4.332X_{153} + 0.431X_{161} + 0.443X_{162}$ with R2 = 0.824.

The value of coefficient of determination (R^2) was found to be 0.8230 which implied that 82.30 per cent of total variation in disease incidence in cotton crop from met weeks 27 to 31 could be accounted for by a linear function involving different weather factors. The Partial regression coefficients, standard errors and proportional contribution of each variable to regression of CLCuD on weather factors are presented in Table 4. Among the weather factors, minimum temperature during current and one prior/lag week and sunshine hours during three prior/lag weeks were found to have highly significant negative influence on disease incidence, while morning relative humidity during three prior/lag weeks, evening relative humidity during

current & four lag weeks and rainfall during first and two prior/lag weeks had significant positive influence. The minimum temperature during current and one week prior period together contributed maximum i.e. to the extent of 41.44 per cent to R^2 where as rainfall during current and two weeks prior/lag period together and morning relative humidity during three week prior period contributed 16.85 and 9.27 per cent, respectively to the total variation in the disease incidence. Evening relative humidity of current and four lag weeks together contributed 9.24% to R^2 .

It was observed that minimum temperature and sunshine hours have significant negative correlation where as morning/evening relative humidity and rainfall have positive correlations with incidence and progress of disease and this regression equation will be helpful in understanding factors affecting disease developemnt and its prediction.

Table 2. Per cent incidence and progress of CLCuD on susceptible cotton variety HS-6 under screening nursery

Year	Per cent disease incidence during different standard meteorological week										
	27*	28	29	30	31	32	33	34	35	36	37
1999	4.15	15.44	15.44	17.44	23.30	27.82	27.82	38.36	40.74	40.74	42.05
2000	33.52	57.19	89.72	95.43	95.43	95.43	95.43	95.43	95.43	95.43	95.43
2001	2.92	15.92	30.98	52.76	77.40	87.42	91.35	94.24	96.76	97.44	98.03
2002	6.16	28.20	42.13	53.13	57.28	59.20	62.78	65.12	68.00	69.18	70.17
2003	1.47	3.20	15.85	25.84	34.29	43.56	52.45	63.90	67.59	68.25	70.48
2004	0.21	0.52	2.10	4.50	8.70	10.80	15.16	16.41	17.65	18.17	18.50
2005	2.3	3.20	8.60	17.20	23.80	29.90	41.70	47.50	55.70	58.60	59.20
2006	4.42	9.77	16.58	39.10	68.00	77.60	86.82	88.85	90.97	92.44	93.82
2007	0	0.00	0.00	0.30	5.11	13.51	22.52	25.22	28.53	32.73	35.44
2008	0	0.00	0.00	0.11	0.22	0.56	0.67	1.68	2.24	2.35	2.69
2009	1.85	2.98	3.83	5.11	12.63	22.98	31.78	42.42	47.52	49.37	50.93

*July 2-8.

Table 3. Population (per three leaves) and progress of whitefly on susceptible cotton variety HS-6 under screening nursery

Year	Whitefly population and its progress during different standard meteorological week										
	27	28	29	30	31	32	33	34	35	36	37
1999	0.24	0.33	0.33	0.33	0.24	0.24	0.24	0.18	0.33	1.00	1.26
2000	0.40	0.47	0.13	0.47	0.42	0.45	0.30	0.80	1.26	1.29	1.40
2001	0.23	0.34	0.37	0.42	0.45	0.42	0.54	0.98	1.60	1.75	2.00
2002	0.05	0.17	0.22	0.53	0.37	0.42	0.35	0.37	0.42	1.00	1.02
2003	0.22	0.29	0.41	0.39	0.37	0.56	0.58	0.80	0.90	1.40	1.30
2004	0.11	0.15	0.07	0.15	0.40	0.57	0.30	0.59	0.62	0.60	0.69
2005	0.11	0.15	0.07	0.15	0.58	0.30	0.93	1.00	1.00	1.40	0.70
2006	0.27	0.69	0.55	0.40	0.29	0.38	0.73	0.69	0.69	1.16	1.58
2007	0.11	0.15	0.22	1.42	0.79	1.47	1.32	0.96	1.26	1.29	1.63
2008	0.05	0.15	0.13	1.11	0.98	0.55	1.07	0.72	0.68	0.70	0.52
2009	1.08	1.67	1.58	1.75	2.08	2.25	2.50	3.33	2.08	1.92	2.25

Table 4. Partial regression coefficients, standard errors and proportional contribution of each variable to regression of disease incidence on weather factors (n = 55)

Variables	Partial regression coefficient	Standard error of partial regression coefficient	t value	Proportional contribution to R2 (%)
X_{120} – Min. Temp. current week	-6.266	1.019	-6.146**	28.39
X_{121} - Min. Temp. one lag week	-3.335	0.937	-3.560**	13.05
X_{133} – RH Morning three lag week	0.683	0.180	3.782**	9.27
X_{140} – RH evening current week	0.552	0.209	2.646*	5.46
X_{144} – RH evening four lag week	0.409	0.180	2.267*	3.78
X_{153} – Sunshine three lag week	-4.332	1.089	-3.979**	5.49
X_{161} – Rainfall current week	0.431	0.133	3.239**	9.43
X_{162} - Rainfall two lag week	0.443	0.159	2.790**	7.42

$R^2 = 0.8230$

*Significant at P = 0.05

**Significant at P = 0.01

Development of disease maps

After the appearance of cotton leaf curl virus disease in a severe form during 2009-10 crop season in some areas of north zone, district level disease development maps were prepared with a view that the information will serve as a baseline for future studies. In Punjab, out of nine cotton growing districts, the disease was very severe in Ferozpur followed by severe in Muktsar and Faridkot and moderate in Moga, Bhatinda, Sangrur and Mansa districts. In Patiala, it was low whereas It was observed in traces in Ludhiana.However, during 2010-11 season the disease was observed to be in severe form from moderate during 2009-10 in Sangrur and Mansa district indicating increased severity. Similarly in Haryana the disease was observed in traces in the major cotton growing districts of Sirsa, Fatehabad, Hisar and Jind whereas it was not observed in other districts like Rohtak, Bhiwani, Jhajjar, Mahindergarh and Rewari. However during 2010-11 season the disease was quite widespread in Haryana and was found to be moderate in Sirsa, Fatehabad and Hisar followed by low in Bhiwani and traces in Rohtak districts. In Rajasthan during both the years, the disease was moderate in Sriganganagar district and low in Hanumangarh. In cotton growing districts located in central part of Rajasthan, the disease was not observed.

Detection of new weed hosts

A number of weeds and other hosts harbour this virus in the off season and serve as main source of primary inoculum for development of disease on cotton. The disease migrates from these plants to cotton crop through its carrier, whitefly. Weeds and hosts from other crops are also present during the season alongside the crop on which the disease keeps on multiplying. A large number of weed hosts reported on the basis of symptoms, transmission studies, ELISA testing and hybridization with DNA A probe have been summarized (Table 5)

Table 5. CLCuV disesase hosts reported from India

Name of host	Type of test	Reference
Sida sps, Abutilon Indicum, Hibiscus rosa sinensis, Althea rosea	Based on visual symptoms	Singh et al.,1994
Phaseolus vulgaris, Capsicum annum, Nicotiana tabacum, Lycopersicum esculentum	Transmission studies and ELISA	Nateshan et al.,1996
Abelmoschus esculentus, Althea rosea, Physalis floridana, Nocotiana benthamiana, Phaseolus vulgaris	Transmission studies	Radhakrishnan et al., 2001
Althea rosea, Sida sps., Ageratum sps., Hibiscus rosa sinensis	DNA-A probe hybridization	Sharma, 2002
Tribulus terrestris, Cucumis sps.	CLCuRv-CPgene and DNA beta amplification	Sivalingam et. al., 2004
Chorchorus acutangularis, Melilotus indica, Ageratum conyzoides	DNA-A & DNA beta probe hybridization	Radhakrishnan et al., 2004
Nicotiana tabacum, Lycopersicum esculentum, Zinnia elegans, Mentha arvensis, Capsicum sps, Hibiscus rosa Senensis, Abelmoschus esculentus, Sida alba	PCR using CP primer	Kang et al., 2004
Sida sps., Achyranthus sps., Clearodeadron sps.	CP gene amplification	Monga et al., 2005
Convolvulus arvensis, Capsicum sps., Pathenium sps., Solanum nigrum, Digeria arvensis, Lantana camara, Achyranthus aspera, Chenopodium album, Spinacea sps., Xanthium strumarium	CP gene amplification	Monga (Personal communication), 2011

Break down of resistance due to development of new viral recombinants

Cotton leaf curl virus disease appeared in a severe form during 2009-10 crop season in north zone. Based on the surveys carried out during the crop season it was observed that the disease was particularly severe in certain pockets of Ferozpur district of Punjab and Sriganganagar district of Rajasthan states. It was also observed that there were severe losses caused due to this disease in some Bt cotton hybrids in Ferozpur district. It was also noted that the earlier known resistant varieties and hybrids in the north zone like F-1861, RS-810, RS-2013, RS-875, LH-2076 and LHH-144 etc also showed symptoms of leaf curl in the above referred hot spot areas. The CLCuD resistant germplasm available in north zone was raised at cotton Research Station, Abohar considered to be a hot spot and showed disease upto varying extent in all the material (Bhatoa et al., 2009).

Regular monitoring of CLCuV affected cotton is done to characterize variability of symptoms, diversity of sequences with in the associated isolates and variability in disease pattern, if, any. Four distinct symptom types were documented viz. leaf curl with prominent enations, severe leaf curl without prominent enations upward and down ward curling of leaves. Sequences of DNA A and ß DNA components of the isolates associated with different symptoms showed existence of significant variation and recombination with other strains of CLCuV. However correlation of specific sequence or recombination event with specific symptom needs to be established. Sequence identity matrix and RDP analysis of DNA A and ß components of six virus isolates analyzed over a period of four years from 2006 showed sequence homology and recombination among several isolates from India and Pakistan. Isolate G6-DC, isolated from cotton cultivar cv. RS 2013, with compromised resistance and severe leaf curl isolate S2 analyzed during 2009-10 showed close resemblance to several CLCuV isolates from Pakistan. DNA A component of G6-DC had major recombination events with two Pak strains, beside other Indian strains while S2 isolate showed major recombination with three Pak strains. Incidentally, Burewala strain was the notorious strain that knocked down resistances of popular varieties at Vehari, Pakistan, in 2002 (Mansoor et al., 2003). These included then highly resistant varieties CIM 448, 443, 446, 473, 435 and FH 900. Accumulation of recombination events over the years coupled with favorable environmental conditions appeared to have knocked down the resistance of cotton during 2009-10 season (Chakrabarty et al., 2010).

Future management strategies

Refinement of screening methodologies and identification of strains based on virulence

The management of disease is possible through development of resistant varieties/hybrids, control of its vector whitefly (*Bemisia tabaci*) and management of alternate hosts including clean cultivation through weed management (Narula et. al.,1999). Among these, development of resistant varieties /hybrids is the most effective and reliable method for which germplasm and new sources need to be properly screened on a regular basis to identify resistant material to be used for varietal/hybrid development. We need to have fool proof cotton leaf curl virus disease screening methodologies for this purpose. At present, Screening under field conditions is carried out at disease hot spots or by using screening nursery and infector rows of susceptible varieties (Monga et. al., 2003; Monga et. al., 2008). Under artificial conditions, screening is carried out through artificial inoculation using viruliferous whiteflies either by free choice method under polyhouse/net house conditions or through release of counted viruliferous white flies on test plants under plastic jars in polyhouse/screen house for fixed interval. The grafting technique however is seldom being used in India. It has been observed over the years that the various techniques require further standardisation/ refinement. For instance the disease development is influenced by several weather, plant and vector factors like temperature, relative humidity, light, plant age, culture and sex of whiteflies etc. These factors affecting disease development need to be understood properly in order to make refinement of techniques.

There has been break down of resistance of hitherto known resistant varieties, of late, and reports on the development of new strains of virus in the region.There is an urgent need to maintain strains collected from different regions based on disease severity and screening of germplasm, varieties and hybrids against different strains will lead to the development of sources of resistance.

Development of transgenics

Transgenic plants derived through exploitation of pathogen derived factor are the most common non-conventional startegy for virus resistance. The RNA interference and post –transcriptional gene silencing has enormous potential in functional genomics and viral silencing. The phenomenon naturaly conserved against kingdoms is thought to protect cells against invasive nucleic acids such as viruses, transposons and aberrant nucleic acids. Most recently ds RNA has been demonstarted to cure infection of DNA viruses like ACMV ,VMYMV,CLCuV and TYLCV besides other retroviral pathogens. For RNA interference mediated resistance against CLCuV, sense and antisense strands of five target sequences of CLCuV located on DNA-A viz. AC2 (150 bp), CP (185 bp), MP (109 bp) and β-DNA viz., βC1 (212 bp) and βV4 (177 bp) were cloned in pBSK-int (3.1 kb), creating five inverted repeat constructs pBSK-int-AC2-SA (3.3 kb), pBSK-int-CP-SA (3.4 kb), pBSK-int-MP-SA (3.2 kb), pBSKint-βC1-SA (3.4 kb) and pBSK-int-βV4-SA of 3.4 kb. The inverted repeat constructs of target viral sequences in pBSK-Int was sub-cloned into pBinAR (11.7 kb) and transformed in *Agrobacterium tumefaciens* strain EHA105. The inverted repeat constructs pBin-CP-S-int-A and pBin-βC4-S-int-A were transformed in *G. hirsutum* cultivar HS 6 by agro-inoculation. Selection of explants was done on MS medium containing 50 mg/l Kanamycin, characterized for the presence of transgene and are being hardened for transfer in green house.Evaluation of dsRNAi mediated transgenic cotton for resistance to whitefly transmitted CLCuV will be done in in glass house.

Vector managemnt

Many efforts have been directed at chemical, cultural and biological control of the whitefly to reduce its numbers. However, eliminating the whitefly entirely as a way of controlling plant virus disease is not practicable because of the fact that even a small population can help in disease transmission and bring about large crop losses.

Another way of managing the disease is control of vector in initial stage by seed treatment with systemic insecticides. This method might prove useful if it is able to protect the plant from virus infection through vector whitefly upto 50-60 days. Even if the infection occurs at a later stage, the severity of losses could be avoided as the symptom development will begin after 65-90 days and the plants will cross the most susceptible stage by that time. Testing of seed treatment chemicals like Imidacloprid 600 FS, Thiomethoxam 70 WS, Carbosulfan 25 DS and Imidacloprid 70WS against CLCuV revealed that CLCuV incidence and whitefly population upto 75 days after sowing in both the years was significantly lower in seed treated plots than untreated control. There was significant reduction in disease and increase in yield at the end of season (Tables 6 & 7) (Singh *et al*., 2002). The use of biopesticide based insecticides during the early part of season is a good alternative for vector management, being safer to natural enemies.

Vector population in the later stages can be minimized by judicious use triazophos @ 1500ml /ha (AICCIP 2009) and Ethion @2000ml/ha (Anonymous 2009). Buprofezin was proved to be effective against nymphs and acetamiprid, diafenthiuron and imidacloprid were effective against the whitefly adults in a study conducted by Ali et. al. (2005). In yet another study the insecticide spirotetramat, applied in mixture with imidacloprid, associated or not with methyled soy oil, was efficient on the control of the whitefly, being viable as a part of the cotton pest management (Papa, et al., 2008). The study on new molecules revealed Acephate 95 SG @526g ai/ha as effective chemistry for the management of whitefly (Anonymous 2010). However, exclusive studies showing management of cotton leaf curl virus disease through its vector whitefly need to be further strengthened.

Table 6. Effect of seed treatment on incidence of CLCuV and white fly population upto 75days.

Treatment ai/ha	CLCuV (%)	W.fly/3 leaves	CLCuV (%)	W.fly/3 leaves
Imidacloprid 600 FS5 ml/kg seed	23.3	1.2	29.4	1.3
Imidacloprid 600 FS 9ml/kg seed	24.7	0.7	29.3	1.6
Imidacloprid 600 FS 12 ml/kg seed	20.9	0.6	24.0	0.5
Thiomethoxam 70 WS 2.8 gm/kg seed	15.6	0.8	16.6	0.7
Thiomethoxam 70 WS 4.8 gm/kg seed	17.8	0.9	19.1	1.0
Carbolulfan 25 DS 50 Gm/kg seed	32.4	1.6	28.4	0.7
Control	40.5	5.5	42.3	2.3
CD at 5%	5.7	0.27	3.7	0.15

Table 7. Relative efficacy of different seed treatment against CLCuV and seed cotton yield

Treatment (ai/ha)	CLCuV %	SC Yield q/ha
Imidacloprid 600 FS 5 ml/kg seed	53.4	11.9
Imidiacloprid 600 FS 9 ml/kg seed	51.9	13.1
Imidacloprid 600 FS 12 ml/kg seed	50.6	11.7
Thiomethoxam 70 WS 2.8 gm/kg seed	42.1	12.8
Thiomethoxam 70 WS 4.8 gm/kg	42.1	13.4
Carbosulfan 25 DS 50 gm/kg seed	56.1	10.6
Control	72.7	5.1
CD at 5%	3.23	10.89

Conclusion

Cotton leaf curl virus disease need to be dealt more seriously in the context of changed scenario leading to the developemnt of recombinants and breakdown of resistance. The new sources of resistance sholud be identified from all available germplasm. The introgression of resistance from other available sources is another option. The development of transgenics using RNAi technology shall go a long way in development of resistance against this important viral disease. Other components of integrated disease management strategy like cultural practices including weed management and vector control can also be pusued vigorously to obtain a holistic approach.

References

Ali, M.A., Rafiq-ur-Rehman, R., Tatla, Y.H and Ali, Z (*2005)* Evaluation of different Insecticides for The control of whitefly on cotton crop in Karor district Layyah.Pak. Entomol.*27:* 5-8.

Ajmera, B.D. (1994). Occurrence of leaf curl virus on American Cotton (*G. hirsutum*) in north Rajasthan. Paper presentation, National Seminar on Cotton Production Challenges in 21st Century, April 18-20 Hisar. India.

Ajmera, B. D., Verma, P. C., Gurjar, K. L. and Pundhir, P. (2004) Sources of resistance to cotton leaf curl virus. Lead paper presented in National seminar on Cotton leaf curl virus disease held at Central Institute for Cotton Resrearch, Regional Station, Sirsa.Published in Proceedings of the National Seminar p 69-71.

Anonymous (1998). Annual Report of All India Co-ordinated cotton Improvement Project for the year1997-98. Pathology report. Coimbatore, Tamil Nadu-641 003.

Anonymous (2005). Annual Report of All India Co-ordinated cotton Improvement Project for the year 2004-05 Pathology report. Coimbatore, Tamil Nadu-641 003.

Anonymous (2006). Annual Report of All India Co-ordinated cotton Improvement Project for the year 2005-06 Pathology report. Coimbatore, Tamil Nadu-641 003.

Anonymous (2009). Package of Practices for Kharif. Directorate of Publication, Punjab Agricultural University, Ludhiana.

Anonymous (2010). Annual Report of All India Co-ordinated cotton Improvement Project for the year 2009-10 Entomology report. Coimbatore, Tamil Nadu-641 003.

Bhatoa , G.S., Sekhon, P.S., Monga D. and Singh, M (2009) Break down of resistance in known *Gossypium hirsutum L.* genetic stocks against cotton leaf curl in north India. Paper presented at 5th International conference on Plant Pathology held at IARI campus, New Delhi from Nov 10-13, 2009.

Chakrabarty, P K, Sable, S, Monga, D and Mayee, C D.(2005) Polymerase chain reaction-based detection of *Xanthomonas axonopodis* pv *malvacearum* and cotton leaf curl virus. Indian Journal of Agricultural Sciences 75 : 524-27.

Chakrabarty, P K., Sable, S., Kalbande, B., Vikas, Monga, D. and Pappu, H.R.(2010) Molecular diversity among strains of CLCuV in north west India and engineering resistance against the disease. Paper presented during the conference of whitefly and thrips transmitted viruses. August 27-28,University of Delhi, South Campus New Delhi, Abstract book, p- 17.

Farquharson, C. O. (1912) A report of the mycologist. A report Agric. Deptt. Nigeria. In M A siddique and L C hungus (Eds) Cotton growth in Gezira environment. W haffer and sons ltd Cambridge England 106p.

Kang, S. S., Athar, M., Cheema, S.S., Malathi, V. G. and Radhakrishnan, G. (2004). Quick detection of cotton leaf curl virus. Indian Phytopath. 57 : 245-46.

Mansoor,S., Amin,I., Iram,S., Hussain, M., Zafar,Y., Malik,K. A and Briddon, R.W.(2003) The breakdown of resistance in cotton to cotton leaf curl disease in Pakistan. New Disease Reports 7: 6.

Monga, D., Narula, A. M. and Raj, S. (2001). Management of cotton leaf curl virus- A dreaded disease in north India. Paper published in Book of papers of National seminar on Sustainable cotton production to meet the future requirement of industry. Organised by Kapas Vikas Nideshalya, Directorate of cotton development, Government of India. Pp112-115.

Monga, D. Raj, S and Mayee, C.D. (2003) Studies on the cotton leaf curl virus disease in north India. Paper published in proceedings of World Cotton Research Conference at Cape Town, South Africa March 9-13, 2003.p 1407-1414.

Monga, D., Kumar, R., Kumar, M. (2005) Detection of DNA A and satellite (DNA beta) in cotton leaf curl virus (CLCuV) infected weeds and cotton plants using PCR technique. J. Cotton Res. Dev. 19: 105-108.

Monga, D., Kumar, M., Chander, S., Singh, N. P. and Meena R. A. (2008) Identification of cotton leaf curl virus disease (CLCuD) resistant lines. J. Cotton Res. Dev. 22 : 234-237.

Monga,D., Alok and Soni, R.(2011) Detection of weeds carrying cotton leaf curl virus disease using PCR.(personal communication).

Narula, A.M., Monga, D., Chauhan, M.S. and Sheo Raj, (1999). Cotton leaf curl virus disease in India-The Challenge ahead. J. Cotton Res. Dev. 13: 129-138.

Nateshan, H.M., Muniyappa, V., Swanson, M.M. and Harrison, B.D.(1996). Host range,vector relations and serological relationships of cotton leaf curl virus from southern India. Ann. appl. Biol.128:233-244.

Papa, G., Furlan, R., Takao, W., Fernando J. Celoto, F.J. and Gerlack,G (2008) Effect of new insecticide (spirotetramat) in mixture with neonicotinoid on the control of whitefly, *Bemisia tabaci* b-biotype (hemiptera: aleyrodidae), in cotton. Beltwide Cotton Conferences, Nashville, Tennessee, January 8-11,1398-1402.

Radhakrishnan, G., Malathi, V. G. and Varma, A. (2001). Novel features of cotton leaf curl virus disease in India. In. 3rd International Gemini Virus Symposium, July 24-28, 2001, JohnInnes Centre, Norwich, Norfolk, U. K., p53.

Radhakrishnan, S., Malathi, V.G. and Varma, A. (2004). Biological characterization of an isolate of cotton leaf curl Rajasthan virus from northern India and identification of sources of resistance. Indian Phytopath.57: 174-180.

Rishi, N. and Chauhan, M.S. (1994). Appearance of leaf curl disease of Cotton in northern India. J. Cotton Res. Dev. 8: 174-180.

Sharma, P. (2002). Molecular approaches for detection and diagnosis of cotton leaf curl Gemini virus and its mode of dissemination in the field. Ph.D. thesis, Department of Plant Pathology, College of Agriculture, CCS HAU, Hisar.

Sivalingam, P. N., Padmalatha, K. V., Mandal, B., Monga, D., Ajmera, B. D. and Malathi, V.G. (2004). Detection of begomoviruses in weeds and crop plants in and around cotton field surveillance: Disease forecasting and management held at IARI, New Delhi , February 19-21,2004. Souvenir and abstract p.36.

Singh, J., Sohi, A.S., Mann, H.S. and Kapoor, S.P. (1994). Studies on whitefly *Bemisia tabaci* (Genn.) transmitted cotton leaf curl virus disease in Punjab. J. Insect Sci. 7: 194-198.

Singh, D., Singh, R. and Garg, H.R.. (2002). Efficacy of different seed treatment chemicals against cotton leaf curl virus. J. Cotton. Res. 16 **:** 40-42.

Tuteja,O.P., Monga, D., Verma, S.K., Meena, R.A., Jeyakumar, P., Singh, P., Kumar, S. and Singh, M. (2005) Shresth(CSHH-198) –A quality cotton hybrid for north zone. J. Indian Soc. Cotton Improv. 30 : 115-119.

Tuteja, O.P., Khadi, B.M., Monga, D., Ahuja,S.L., Verma,S.K., Meena,R.A., Jeyakumar, P and Kumar,S. (2006) CSHH-238-A high yielding hybrid cotton for north zone. J. Indian Soc. Cotton Improv. 31 : 105-111

Tuteja, O.P., Khadi, B.M., Monga, D, Verma,S.K., Ahuja,S.L., Meena,R. A., and Kumar,S (2009) CSHH-243-A quality cotton hybrid for north zone. J. Indian Soc. Cotton Improv. 34 : 38-41.

Varma, A., Puri, S.N., Raj, S., Bhardwaj. R.P., Kannan, A., Jayaswal, A.P., Srivastava, M. and Singh, J. (1995). Leaf curl disease of cotton in North-West-India. Report of the ICAR Committee, September, 1995.