



## Elite sources of resistance against Cotton Leaf Curl Virus disease under high inoculum pressure in the field and using graft inoculations in American cotton

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Cotton is the most important *kharif* season cash crop of north India. Among the various factors responsible for its low production and productivity during the last two decades, cotton leaf curl virus disease (CLCuVD) 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 northern India comprising an area of around 15 lakh ha. 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* L.) 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) on *hirsutum* cotton and posed a major threat to its cultivation in northern India (Varma *et al.* 1995). The disease appeared in an epidemic form during 1997 in the Rajasthan and in Punjab affecting an area of 0.1 million ha (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 (Anonymous 2011). The hitherto known resistant varieties also showed susceptible reaction at hot spot areas.

In Indian subcontinent (Pakistan) the disease was first reported in late 1960s and remained a minor sporadic problem till 1980's. From 1992 to 1997, this disease appeared in very severe form and affected Pakistan economy up to US \$ 5.0 billion (Radhakrishnan *et al.* 2004). The first symptoms of disease on cotton in Punjab appeared in 1995 and the disease continued to spread steadily eastwards in Punjab, Rajasthan and Haryana states (Briddon 2003). The incidence of CLCuD varied from traces to 10; traces to 30 and 10 to 80 % in the month of June, July and August respectively on different varieties /hybrids in various cotton growing areas of the Punjab in 1998 and 1999 ( Singh *et al.*

2001). From *kharif* 2005 to *kharif* 2010 cotton season, the incidence of CLCuD varied from traces to 8% along with I to III grade severity on different varieties/hybrids viz. F 1861, LHH 144, MRC 6304 Bt and F 1378 at different farmers field, research farms of Bathinda, Faridkot, Muktsar and Ferozepur districts. The incidence/severity of CLCuVD increased from June to September in *kharif* 2005, 2006, 2007, 2008, 2009 and 2010 crop seasons in different cotton growing areas of the state. The maximum incidence and severity of CLCuVD was observed in *kharif* 2009 and 2010 on various hybrids/varieties of American cotton at farmers fields and at research farms. In *kharif* 2010, it appeared in epidemic form in almost all the varieties/hybrids of the cotton. The incidence was traces to 65.0% with 0-IIIrd grade symptoms ( MRC 7017 and RCH 134 Bt); traces to 100.0% with 0-IVth. grade symptoms (RST 9 and RCH 134 Bt); traces to 95.0% with 0- IVth. grade symptoms (F 846 and RCH 134 Bt) in Bathinda, Faridkot, Muktsar and Ferozepur area. (Anonymous 2006-2011). Due to breakdown of resistance from 2011 onwards (Bt hybrids like MRC 7017 BGII , MRC 7031 BG II and Anukar 3028 BGII) till date many of Bt hybrids tested in research trials in north zone of India showed susceptibility/tolerance to CLCuVD. At present major cotton area is under BG II cotton hybrids which are tolerant but none is resistant to CLCuD (Anonymous 2015).

Most of the begomoviruses comprised of two genomic components called DNA-A and DNA-B, which are indispensable for the disease that is transmitted by whitefly *Bemisia tabaci* (Monga *et al.* 2011). Population build up of whitefly over the years has taken place, but there is no relation of vector population with CLCuVD incidence and severity. During 2015-16, there was whitefly epidemic but leaf curl incidence is traces to low. It is the most vital sucking pest of both industrial and food crops like cotton, sunflower, melons, tomato, brinjal etc. (Rafiq *et al.* 2008). It is polyphagous in nature and is confirmed over 500 plant species all over the world (Greathead 1986). In cotton growing areas of central Punjab it has been reported in about 164 plant species (Attique *et al.* 2003). In 16 of the 27 cotton growing countries whitefly is recognized as a

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major pest during mid to late sowing time. The variability of DNA beta component of CLCuD causative complex has been established and the sequence has been submitted to NCBI, viz. KJ 614434, KJ 614435 and KJ 614436 (Inder *et al.* 2014).

Losses due to CLCuD are dependant on infectivity time and variety. The pronounced damage of CLCuD is at early stage of infection but at later stages results minor infections (Akhtar *et al.* 2003). CLCuD damage differs on various plant parts and ultimately results in reduction of yield. It can reduce boll weight 33.8%, 73.5% in bolls per plant, GOT% upto 3.93%, seed index 17.0% and yield per plant 64.5% (Ahmed 1999). The cotton fiber (lint) is the most important commodity for textile industry and CLCuD also affects fiber quality traits (Kalhor *et al.* 2002). According to Ahmed *et al.* (1999), CLCuVD can decrease fiber length by 44%, fiber strength by 10% and elongation percentage upto 10%. Akhtar *et al.* (2009) studied impact of CLCuD on fibre quality traits and the findings depicts that the CLCuD significantly affect traits like GOT, fibre length, fibre uniformity index, short fiber index, fibre fineness, fibre bundle strength yellowness and maturity ratio. In their studies they observed significant affects of this viral disease on cellulose, protein, wax and pectin which are the major constituents of fibre. But in view of Idris (1990) virus has significant impact on yield but not on fibre quality. Singh *et al.* (2002) reported that CLCuD caused 64.7% reduction in number of bolls along with 49.6% reduction in boll weight in susceptible variety F 846. It was also found that this disease remarkably deteriorated the quality of fibre, i.e. fibre length reduced by 2.9%, elongation by 13.0%, uniformity by 1.6% and micrinare value by 6.3% in diseased plants having grade 1, 2, 3 and 4 symptom of disease over 0 grade (healthy plants).

Use of resistant varieties/hybrids remains the most viable, environmentally safe, ecologically sound and also less expensive method for the management of this dreaded disease. Therefore the present investigations were undertaken to find out the elite sources of resistance in different varieties/hybrids of American cotton. During the past 20 years, there have been considerable efforts to develop CLCuD-resistant cultivars in India. Up until recently, the disease was efficiently managed by developing CLCuVD-resistant varieties. This study was initiated to identify sources of resistance in cultivated genotypes/hybrids representing *Gossypium hirsutum*.

#### Field screening

The experiment was conducted at Punjab Agricultural University, Regional Station, Faridkot and Cotton Research Station, Abohar (Punjab) India, in *kharif* 2009, 2010 and 2011 seasons to identify resistant entries against CLCuVD, under natural epiphytotic conditions. During three years 43, 45 and 47 varieties/hybrids respectively were sown in 12.0 meter row length at a spacing of 67.5 × 60.0 cm (for variety) and 67.5 × 75.0 cm (for hybrid). Three lines of a susceptible variety, RS 921, were grown as border

Table 1 The varieties/hybrids categorized against Cotton Leaf Curl Viral Disease

Symptom	Disease severity	Disease index %	Disease response
Complete absence of symptoms	0	0	Immune
Thickening of few small scattered veins on one or few leaves of a plant observed after careful observations.	1	0.1-10	Highly resistant
Thickening of small group of veins, no leaf curling, no reduction in leaf size and boll setting.	2	10.1-20	Resistant
Thickening of all veins, minor leaf curling & deformity of internodes with minor reduction in leaf size but no reduction in boll setting.	3	20.1-30	Moderately resistant
Severe vein thickening, moderate leaf curling followed by minor deformity of internodes and minor reduction in leaf size and boll setting.	4	30.1-40	Moderately Susceptible
Severe vein thickening, moderate leaf curling & deformity of internodes with moderate reduction in leaf size and boll setting followed by moderate stunting.	5	40.1-50	Susceptible
Severe vein thickening, leaf curling, reduction in leaf size, deformed internodes and severe stunting of plant with no or few boll setting	6	>50	Highly susceptible

$$\text{Disease index (\%)} = \frac{N1 S2}{S1 N2} \times 100$$

N1, Number of plants in check; N2, Number of plants in test entry; S1, Sum of all grades in check; S2, Sum of all grades in test entry

row (nursery plant). One row of the susceptible check was also grown after each test entry. Observations regarding incidence /severity of the disease were recorded from the appearance of the disease from i.e. first week of July upto end of October in *kharif* 2009, 2010 and 2011 at 15 days intervals. Based on new scale (Anonymous 2009) the varieties/hybrids were categorized against cotton leaf curl viral disease (CLCuVD) as given in Table 1.

#### Graft inoculation evaluation

The artificial screening against CLCuVD was also carried out by grafting in *kharif* 2012 and 2013. Twig and petiole grafting as reported by workers from Pakistan (Akhtar *et al.* 2002). The plants of CLCuVD susceptible

variety RS 921 were raised in glasshouse under insect free conditions at temperature of  $33 \pm 2^{\circ}\text{C}$  and average relative humidity (Morning-66%, Evening-48%). In this technique, 6 week old plants were diagonally cut 2-3 inches below the growing tip. An actively growing CLCuVD branch of similar thickness (variety RS 921) raised in poly house was collected and a similar diagonal cut was made on it. The cut portions were joined together and tied firmly with parafilm. The organic connection was established and a new branch emerged below the graft showing CLCuVD symptoms on new emerging leaves as early as on fifth day after grafting. The successful transmission of CLCuVD was 91.7% in petiole grafting, 83.3% in twig grafting and 100% in the new technique, the appearance of symptoms was noticed from 5<sup>th</sup> day onward and all the tested plants showed symptoms within three weeks. Whereas, in case of twig and petiole grafting, the symptoms appearance was noticed between 11-26 days and 17-30 days respectively. The same procedure was adopted for the conduct of experiment in both the years. Finally the same rating scale was used for categorization of entries against CLCuVD as under field conditions based on two year observations of glasshouse.

CLCuVD is presently a serious constraint in cotton production across Pakistan and north-western India (Ullah *et al.* 2014). Very limited sources of resistance/tolerance in cotton germplasm against CLCuVD are available. Present study reports a comprehensive evaluation of levels of resistance/ susceptibility to CLCuVD in number of cotton genotypes for the first time under high inoculum pressure at two hot spot locations under field conditions and using grafting in the glasshouse.

#### *Evaluation of cotton genotypes in field under high inoculum pressure*

In *kharif* 2009, out of 43 varieties /hybrids one variety, i.e. F 2228 showed moderately resistant reaction at both the locations. Similarly in *kharif* 2010, one variety namely F 2276 exhibited moderately resistant reaction against the disease at both the locations. In *kharif* 2011, variety RS 2670 showed highly resistant reaction; F 2310, GSHV 162 and LH 2076 showed resistant; HS 289 and H 1442 gave moderately resistant reaction against CLCuVD at both the locations. LHH 144 exhibited moderately resistant to highly resistant reaction against CLCuVD. Whiteflies started to appear in low densities just after the emergence of seedlings and continued to their build up during the whole growth period of cotton during 2009-2011 at both the locations. The first disease symptoms were seen as thickening of small veins and curling of leaves on few young leaves of a small number of plants after 30-35 days of sowing in all three years of testing. PDI increased with the passage of time depending upon the genetic makeup of genotypes. Plants infected at an early stage of growth exhibited severe symptoms, while mid season and late infections were milder and expressed reduced symptoms. Severe symptoms started with a minor vein thickening of young leaves which

progressed to severe vein thickening, leaf curling, reduction in leaf size, deformed internodes which culminated in severe stunting of the plants with no or few bolls. The genotypes showing resistant to tolerant response during one year became moderately susceptible to highly susceptible in the next year. These results are in accordance with the reports of Akhtar *et al.* (2010) who performed the screening of large number of non-Bt-cotton genotypes belonging to *G. hirsutum* species against CLCuVD under field and found the same type of trends.

These results are also in conformity with the findings of Singh *et al.* (2000) who found that hybrid LHH 144 was highly resistant against CLCuD. Similarly, Singh *et al.* (2001) reported that hybrid LHH 144 was highly resistant against the malady under natural epiphytotic conditions. Yadav and Singh (2003) screened 100 germplasm lines/ hybrids under natural epiphytotic conditions in the field and recorded that hybrid LHH 144 was free from the disease. Further, Singh (2003) also reported that hybrid LHH 144 was free from the disease. A variety F 2036 and hybrid LHH 144 showed resistant reaction against CLCuVD under natural epiphytotic conditions (Singh *et al.* 2006, Singh *et al.* 2008). Therefore, these moderately resistant/ resistant lines can be used in the breeding of agronomically improved varieties/hybrids of cotton.

#### *Evaluation of cotton genotypes using graft inoculation*

Results of the evaluation of cultivated *G. hirsutum* genotypes by graft inoculation with CLCuVD (based on the pooled data of *kharif* 2012 and *kharif* 2013) showed that all the tested genotypes were moderately resistant to highly susceptible with an average PDI of 22.9 to 100% within 35–45 days of grafting and displayed generalised and pronounced symptoms along with 10-14 days latent period. Inoculated plants exhibited severe disease within 10 days after the appearance of initial symptoms, whereas enations were observed after 25 PDI, and no reduction in severity index was observed until the end of the experiment, that is, 90 days after grafting. However, through graft evaluation none of the graft inoculated plant was symptomless. All the tested entries showed 22.9 to 100.0% disease index and latent period was 10 to 14 days. Out of 43 entries evaluated using graft inoculation in glass house, three entries namely MR 786, F 2228 and LHH 144 showed moderately resistant reaction to CLCuVD. Rest of entries showed moderately susceptible to highly susceptible reaction to CLCuVD. Akhtar *et al.* (2010) also reported that through grafting no source of resistance was found in non-Bt-cotton genotypes belonging to *G. hirsutum* species.

A successful plant breeding programs for disease resistance depends on the effective identification of sources of resistance and accuracy in resistance assessment technique (Akhtar *et al.* 2010, Pico *et al.* 1998). The current results obtained through natural transmission of virus by vector whitefly under natural field conditions and artificial inoculations through grafting under glasshouse conditions showed that the disease response of Bt-cotton genotypes varied

according to the virus transmission method employed. Field screening is a routine procedure and cannot be treated as a reliable procedure since it is seasonal and depends on epidemic conditions and may produce misleading results as has been evident from current findings. On the basis of present findings it can be concluded that graft inoculation is the most suitable, efficient and adequate method, and deemed practical in terms of screening. However, the field screening does not discriminate between these levels but can serve as a first step for further studies using graft inoculation.

#### SUMMARY

The pooled data of both the locations in three years showed that in *kharif* 2009, out of 43 varieties /hybrids one variety of American cotton (*Gossypium hirsutum* L.), i.e. F 2228 showed moderately resistant reaction against Cotton Leaf Curl Virus Disease on both the locations. Similarly in *kharif* 2010, one variety F 2276 exhibited moderately resistant reaction against the disease at both the locations. In *kharif* 2011, variety RS 2670 showed highly resistant reaction; F 2310, GSHV 162 and LH 2076 also showed resistant reaction; HS 289 and H 1442 gave moderately resistant reaction against CLCuVD at both the locations respectively. LHH 144 exhibited moderately resistant to highly resistant reaction against CLCuVD. However, through graft evaluation in *kharif* 2012 and 2013, the pooled result of both the years indicated that none of the graft inoculated plant was symptomless. All the tested entries showed 22.9 to 100.0% disease index and latent period was 10 to 14 days. Out of 43 entries evaluated using graft inoculation in glass house in *kharif* 2012 and 2013, three entries viz., MR 786, F 2228 and LHH 144 showed moderately resistant reaction to CLCuVD. Rest of entries showed moderately susceptible to highly susceptible reaction to CLCuVD in both the years respectively. It was suggested that these resistant varieties/hybrids could be further used in breeding programme, to develop high yielding, CLCuVD resistant and good fibre quality varieties/hybrids of American cotton in the northern zone of India.

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