Indian Journal of Agricultural Sciences **87** (5): 681–4, May 2017/Article
https://doi.org/10.56093/ijas.v87i5.70199

Cotton leaf curl virus disease (CLCuVD) predictive model based on environmental variables

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Received: 14 June 2016; Accepted: 04 November 2016

ABSTRACT

Cotton (*Gossypium* spp) is one of the most important cash crops in India. The productivity of cotton in the last decade has suffered a great set back due to Cotton leaf curl virus disease (CLCuVD) in Indian Punjab. It has devastated cotton production during the past couples of decades or so causing serious problems in its management. This study, therefore, was initiated to develop a disease predictive model to predict epidemiological factors conducive for disease spread/ incidence. Four years data of CLCuVD incidence, whitefly population density, and environmental variables were collected for the development of a predictive model from the experiments conducted at Punjab Agricultural University, Regional Station, Faridkot, Punjab (India). A close relationship was observed between CLCuVD incidence and whitefly population. A predictive model based the on data (2010-2013) of CLCuVD incidence, whitefly population density, and environmental variables was developed ($Y=253.1-11.8^{*}$ Min T + 3.49 Max T+0.682^{*} Min RH-1.13^{*} Max RH-0.20 RF +1.65^{*} WF popn. ; $R^2=0.62$). The model so developed was validated for the year (2014). Minimum temperature has significant negative and minimum relative humidity along with vector has significant positive, contribution towards the appearance of disease. So if the minimum temperature in the months of June and July is less than 26^0C to 28^0C and minimum relative humidity is more than 50%, then chance of appearance of CLCuVD is maximum. There was a moderate difference between the slope of observed and predicted values (5.85 and 6.68) with R^2 of 0.82 and 0.78, respectively. It was envisaged that the model would be helpful in forecasting the disease to decide the correct timing of pesticide application, in order to manage CLCuVD effectively.

Key words: CLCuVD, Punjab, Rainfall, Relative humidity, Temperature, Whitefly

Cotton leaf curl virus disease has great importance. It was reported first in Nigeria (1912) on *Gossypium peruvianum* and *Gossypium vitifolia*, Sudan (1924), Tanzania (1926), Philippines (1959). The first evidences of CLCuVD on the Indian subcontinent was observed in the vicinity of the city of Multan, Pakistan in 1967 (Aslam and Elahi 2000). It remained a minor sporadic problem for the following two decades. This malady has been estimated to have affected on the economy of Pakistan up to US $ 5.0 billion from 1992 to 1997 (Briddon and Markham 2001). In India, CLCuVD was first observed affecting cotton in 1989 at Indian Agricultural Research Institute, New Delhi. The disease was also observed in farmers fields in Sri Ganaganagar and adjacent districts bordening Pakistan (Rishi and Chauhan 1994). In 1994, the cotton production in Sri Ganganagar and its adjacent districts in Indian Punjab were severely affected by an epidemic of CLCuVD. Slowly and steadily, the disease spread throughout cotton growing areas from 1994 to 1998, due to the mobility of the vector (Monga *et al.* 2004).

CLCuVD is a major limitation to cotton production in the Indian subcontinent. A study based on sequence analysis of virus genome showed that the resistance breaking cotton leaf curl Burewala virus (CLCuBuV) is now dominant virus in many fields. In northern India 10 million ha areas under cotton is spread over the states of Punjab, Haryana and Rajasthan which includes 16% of the total cultivated land where cotton is grown, and contributes towards 20% of the total cotton production in India. CLCuVD affects vast areas under cotton cultivation and is limiting factor in cotton production (Singh *et al.* 2010, Monga *et al.* 2011). Once affected by the virus, the plants show upward and downward curling with thickening of veins, enations on the abaxial surface, overall stunting, flower and fruit abortion leading to low productivity. CLCuVD is caused by a complex of viruses that belong to the genus *Begomovirus*, family *Geminivirdae*, commonly referred to as CLCuVD–associated begomoviruses (CBVs). CBVs, are common, with the majority of Old World begomoviruses monopartite, with genomes that consist of only one circular single-stranded DNA (ss DNA) molecule and are associated with beta satellite (Briddon *et al.* 2008) and , frequently, a third component known as alphasatellite (Amrao *et al.* 2010). The CBV complex is transmitted by the whitefly *Bemisia*

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tabaci (Genn.) , referred to as a sibiling species group.

In 2001-2002, a second CLCuVD epidemic occurred in Pakistan, resulting in 100% crop losses in many areas. This outbreak was atributed to a new, virulent begomovirus that emerged from the cotton fields in the vicinity of Burewala in Punjab district of Pakistan and was named by the Burewala strain. The virus associated with this epidemic is now recognized as a distinct begomovirus–cotton leaf curl Burewala virus (CLCuBuV) (Amrao *et al.* 2010). This is the resistance –breaking strain of the virus that has been shown to infect all of the previously known resistant and tolerant varieties of the cultivated *Gossypium hirsutum* in India and Pakistan (Anonymous 2015, Akhtar *et al.* 2010).

Depending upon the stage of crop and the time of infection the losses in seed cotton yield varied in India from 10.5 to 92.2% in the susceptible varieties of upland cotton (Singh *et al.* 1995, Monga *et al.* 1998). In Pakistan CLCuD causes 33.4% reduction in boll weight and 72.3% in the number of bolls in susceptible variety S 12. Due to CLCuD infection ginning out turn (GOT) was reduced by 3.8%, seed index by 17.3%, fibre length by 3.9%, strength by 1.0% and elongation up to 10.0% (Ali Mahbub *et al.* 1995). During 2009-2010, severe CLCuVD symptoms were observed in cotton fields in Bathinda, Abohar, Fazilka of Punjab and Sri Ganganagar of Rajasthan state, causing yield loss up to 100%. Cotton varieties and hybrids that were previously resistant to CBVs also developed severe symptoms (Rajagopalan *et al.* 2012). A single viruliferous whitefly can successfully transmit this disease (Yassin and El-Nur 1970). Thus, the intensity of the disease incidence could not be correlated with whitefly population. However, the same variety was not affected similarly in all the areas or within an area. Its management can be achieved by adopting various strategies well in advance (Monga *et al.* 2004). The plant to plant difference in exhibiting the virus symptoms appears to be of environmental nature. As the disease incidence and its progress depends upon host pathogen environment and vector interaction and considering the importance of the disease in terms of yield losses and quality characters, the present investigations were undertaken, with the objective to determine environmental conditions conducive for the development of CLCuVD in major cotton–growing areas of the Punjab, India. To develop a disease predictive model to establish a forecasting system in future will provide a base to take preemptive decision against the insect vector and CLCuVD under a given set of environmental conditions for better management practices.

MATERIALS AND METHODS

The experiment was conducted for four consecutive years from 2010 to 2013 at Punjab Agricultural University, Regional Station, Faridkot, Punjab (India) with leaf curl susceptible variety-F 846, the crop was sown with row to row spacing of 67.5 cm and plant to plant spacing of 30.0 cm in a plot of 270 m^2 . All the recommended packages of practices were followed. Observations on the appearance and progress of CLCuD incidence (from each plant), along with whitefly population per three fully developed leaves from 20 randomly selected plants were taken at weekly intervals starting with the onset of disease. The data were taken untill the occurrence of maximum disease incidence, for each year. The daily data regarding maximum and minimum temperature, maximum and minimum relative humidity and rainfall were also recorded at meteorological observatory of Punjab Agricultural University, Regional Station, Faridkot (Punjab), India. Average of meteorological week basis CLCuVD (%), whitefly population along with environmental variables were calculated. The detection of virus was confirmed through Polymerase chain reaction (PCR). The influence of each environmental variable (minimum and maximum air temperature, rainfall, and relative humidity) on CLCuVD was determined by regression analysis. All data analysis was carried out using MINITAB software.

For molecular diagnostics DNA was extracted from leaf samples using the CTAB method (Doyle and Doyle 1990). Viruses and betasatellites were detected in DNA extracts by PCR with primer pairs CLCV1/CLCV2 (5′CCGTGCTGCTGCCCCCATTGTCCGCGTCAC3′/5′CTGCCACAACCATGGATTCACGCACAGGG-3′) and Beta01/Beta02 (Briddon *et al.* 2002), respectively.

RESULTS AND DISCUSSION

CLCuVD predictive model based on 4 years data (2010–2013)

In the present study, a multiple regression model was developed. The statistically justified model ($R^2 = 0.62$) at $P < 0.05$ developed to predict the probable attack of CLCuVD under a given set of environmental variables is: ($Y=253.1-11.8^{*}\ x_1 + 3.49\ x_2+0.682^{*}x_3-1.13^{*}x_4-0.20\ x_5 +1.65^{*}\ x_6$ with $R^2 = 0.62$, where Y = CLCuVD, x_1=minimum temperature, x_2 = maximum temperature, x_3 = minimum relative humidity, x_4 = maximum relative humidity, x_5 = rainfall, and x_6 = whitefly population. It is evident from the model that major factors responsible for the attack of CLCuVD were minimum temperature, relative humidity and the extent/intensity of whitefly population prevalent at that time. It indicated that with one unit change in minimum temperature, there would be a probable change of 11.8 units in CLCuVD. The change would be 0.682 units in the case of minimum relative humidity. Whitefly, considered as a vector in the onset of CLCuVD, has a significant contributing role in the spread of this viral disease, i.e. linear expansion in a single part of whitefly would cause an equivalent swell in 1.65 units of CLCuVD.

Model assessment

One of the most important parameters to check the model reliability is the value of coefficient of determination, i.e. R^2. In the present study, it was 0.62 which is considered fairly good particularly under field conditions (Table 1). Similarly, the standard error of estimate was not so high (15.5). The F distribution of the regression model was significant at $P < 0.05$ (Table 2). The relative contribution

Table 1 Regression statistics of the predictive model for CLCuVD based on 4 years (2010-2013) data

Regression statistics	
R-square	0.62
Adj-R square	0.57
Standard error	15.5
Observation	60

of minimum temperature, minimum and maximum relative humidity, and whitefly towards the development of model was significant at < 0.05, except rainfall. It may be concluded that the model is good for prediction/forecasting purpose from a set of unknown variables based on the physical theory.

Table 2 Analysis of variance of the predictive model for CLCuVD based on 4 years (2010-13) data

Source	DF	SS	MS	*F*	*P* value
Regression	6	39843.8	6640.6	14.09	<0.001
Residual error	53	24,966.2	471.1		
Total	59	64,810.1			

** *P*<0.001

CLCuVD model validation

The most reliable option in validating the model is making a prediction by using data from other year, i.e. any other data not used in the model development. Therefore, the model so developed on 4 years data (2010-2013) was validated on the data of year 2014. It was observed that there was a moderate difference between the slope of observed and predicted values (5.85 and 6.68) with R^2 of 0.82 and 0.78, respectively (Fig 1).

These findings confirm the results of Bink (1975) who reported environmental factors, viz. temperature and wind etc. affected the epidemiology of leaf curl in Africa. In Pakistan the infection of CLCuVD in eight of the varieties of American cotton was observed at maximum and minimum temperature of 33-45 ^{0}C and 25-30 $^{\circ}$C respectively (Khan *et al.*1998). Singh *et al.* (2003) also found that CLCuVD (%) was significantly correlated with minimum, maximum and mean temperature; minimum, maximum and mean relative humdity, rainfall and whitefly population in particular seasons. Monga *et al.* (2004) made prediction equations for the appearance of CLCuVD. They found that a maximum temperature between 35-42^{0}C, minimum temperature between 26-29 ^{0}C and maximum relative humdity 71-95% in the month of July *in kharif* 2002 and 2003 seasons favoured the maximum development of the disease.

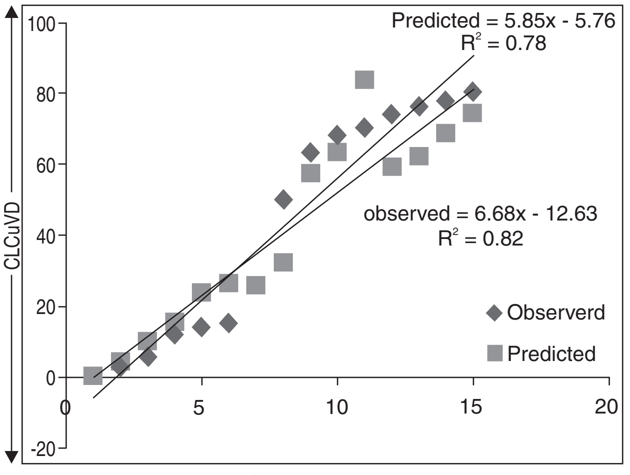

Fig 1 A comparsion of predicted and observed data points based on 4 years data

Singh *et al.* (2005) predicted CLCuVD under disease prone areas using linear model. They reported that minimum temperature (27.3-32 $^{\circ}$C), mean temperature (30.9 - 35.2 $^{\circ}$C), minimum relative humdity (47.5 –63.5%) and mean relative humdity (55.5- 71.0%) in the month of July favoured the maximum development of the disease. Minimum and relative humidity were the major factors which played most important role in the appearance of the disease. Singh *et al.* (2010) gave prediction of CLCuVD in disease prone area using linear model. They reported that minimum temperature (27.3-32.0 ^{0}C), minimum relative humidity (47.5 -63.5%) and mean relative humidity (55.5-71.0%) of July favoured the appearance of the disease. In the present investigations also, minimum temperature and relative humidity played very important role in the appearance and progress of the disease. Monga *et al.* (2011) found that minimum temperature showed significant negative correlation, whereas morning relative humidity gave positive correlations with incidence and progress of the disease and this regression equation helped in understanding the factors affecting disease development and its prediction. Farooq *et al.* (2014) studied that climate change and weather fluctuation have profound influence on the spread of Cotton leaf curl virus disease . Further it was observed that temperature and plant age influenced the Cotton leaf curl virus disease epidemiology. Similarly Khan *et al.* (2015) reported that the CLCuVD appearance directly linked with rainfall and humidity. The third most influential factor of increased CLCuVD incidence was the vector population, which was also strictly dependent upon monthly mean temperature of the particular area.

The minimum temperature and minimum relative humidity were identified as main environmental variables contributing to the development of disease. The influence of rainfall was either nil or meager with respect to disease development. A predictive model ($Y=253.1-11.8^{*} x_1 + 3.49 x_2+0.682^{*} x_3-1.13^{*}x_4-0.20 x_5 +1.65^{*} x_6$ with $R^2 = 0.62$) statistically justified at $P < 0.05$ based on 4 years data (2010-2013) was developed which was then calibrated and validated using data for 2014 . The forecasting accuracy of the predictive model was considerably better, $R^2 = 0.78$, significant at $P < 0.05$. The model indicated a good fit between observed and simulated data. Therefore, farmers and agriculture experts are advised to keep an attentive eye on temperature fluctuations in their area. Timely application of vector control measures will lead to the reduced spread of disease and ultimately lesser damages. The model can very well be used for timing management operations in order to get maximum yields of cotton even in the disease epidemic areas.

ACKNOWLEDGEMENT

The authors are thankful to Director, PAU, Regional Station, Faridkot and ICAR, New Delhi for providing the required research facilities is duly acknowledged.

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