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SCREENING OF COTTON GENOTYPES FOR RESISTANCE TO COTTON LEAF CURL DISEASE UNDER NATURAL INOCULATION CONDITIONS IN SINDH, PAKISTAN

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ABSTRACT

Cotton leaf curl disease (CLCuD), caused by Begomovirus(es), is an economically significant disease affecting cotton crops in Pakistan. The incidence of the disease can reach up to 100% under favorable environmental conditions. While yield losses of up to 30% have been reported due to CLCuD in Pakistan, no efficient management strategy for this disease has been introduced in the region. Management of CLCuD using natural sources of resistance is a long-term strategy of great significance. However, the emergence of new isolates of the viruses can break down the resistance in existing genotypes. Testing new and already available genotypes for resistance to the disease is therefore important to prevent the distribution of susceptible hosts on a large scale. In the present study, we evaluated the resistant potential of 33 cotton genotypes in the agro-ecosystem of Sindh, Pakistan, during the growing season of 2018 and 2019 in three repeated experiments. Observations were taken at intervals of 30 days after sowing (DAS) on the incidence and severity of CLCuD under field conditions. The disease index was then calculated from the values of initial observations on the incidence and severity of the disease at each time point. Finally, the Area Under the Disease Progress Curve (AUDPC) was calculated for the disease incidence, severity, and index. In this study, we highlight the semi-quantitative estimate of the disease through the index values. There was a significant difference among the AUDPC values of the disease index of the tested genotypes (DF=32, F=73.25, P=0.0000 and DF=32, F=225.88, P=0.0000) during 2018 and 2019, respectively. The significantly highest AUDPC value was recorded in BT.CIM-678 (664109.0), followed by CRIS-585 (277732.0), CRIS-613 (133917.0), and CRIS-522 (159272.0) during 2018. The remaining genotypes gave significantly lower AUDPC values. Similar results were obtained during the next year of the experiment. Therefore, it is concluded that the cultivation of BT.CIM-678, CRIS-585, CRIS-613, and CRIS-522 should be avoided in the agro-ecosystem of Sindh. Further studies should be conducted to develop eco-friendly management strategies for the disease.

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INTRODUCTION

Cotton (Gossypium hirsutum) is a significant cash and fiber crop in Pakistan, contributing approximately 8.6 percent to agriculture and 1.8 percent to the country's GDP (Azhar *et al.*, 2013; Farooq *et al.*, 2014). In 2020, cotton was cultivated on 2,078,899 hectares in Pakistan, producing 3,454,334 tons with an average yield of 16,616 kg/hectare (FAO, 2021). However, the yield of cotton per acre has decreased in recent years due to various factors, including attacks of diseases, insect pests, weeds, and environmental factors.

Cotton leaf curl disease (CLCuD), caused by Begomovirus, is a significant factor that affects the various yield and fiber traits of cotton (Farooq *et al.*, 2011). In Pakistan, the biological yield of cotton crops is typically reduced by up to 30% due to the influence of CLCuD (Ashraf *et al.*, 2013; Hassan *et al.*, 2013). CLCuD was first reported in Nigeria in 1912 and gradually spread to Tanzania in 1926 and Sudan in 1934. In Pakistan, it was first reported in the Multan district in 1967 (Hussain and Ali, 1975), and it re-emerged in 1988, severely affecting the newly developed S-12 variety (Briddon, 2003). CLCuD is transmitted from infected plants to healthy ones by the whitefly (*Bemisia tabaci*) through a persistent circulative non-propagative manner (Akhtar *et al.*, 2002). Initially, the thickening and yellowing of small veins on the underside of the leaves are usually observed in diseased cotton plants. Later on, symptomatic leaves may curl downward or upward, and due to the reduction in the distance between internodes, the plant shows stunting (Briddon *et al.*, 2001; Qazi *et al.*, 2007). Plants under severe attack of the disease may show enation, which looks like a leaf and is produced on the underside of the leaf (Mansoor *et al.*, 1993; Harrison *et al.*, 1997). Infection of the virus at an early growth stage may severely delay the flowering, boll formation, and maturation, as well as the quantity and quality of the fiber (Rehman *et al.*, 2019; Monga *et al.*, 2011).

Various management practices are employed to combat Cotton Leaf Curl Disease (CLCuD), with Rahman *et al.* (2017) categorizing these as short-term practices aimed at reducing vector populations and long-term management involving the use of resistant varieties. The development of resistant varieties remains a crucial long-term approach to mitigate the devastating effects of CLCuD (Akhtar *et al.*, 2002). Therefore, screening genotypes for resistance to CLCuD is necessary to minimize exposure of susceptible genotypes in the field and to incorporate such resistance sources into different breeding programs.

In recent studies, many researchers have evaluated various cotton varieties/genotypes for resistance to CLCuD. Batool *et al.* (2021) reported that FH-490, FH-444, FH-458, VH-363 and varieties such as FH-142, NIAB-878, FH-Lalazar, VH-327 exhibited very good results against the virus. Wazeer *et al.* (2020) identified MNH552, CIM-506, CIM-443, CIM-448, CIM-446, MNH-886 and IR-3701 as resistant varieties, while BH-36, RH-112, CIM-240, CIM-70, MNH-329, MNH-147, BH-186 and BH-175 were found to be susceptible to CLCuD. Naveed *et al.* (2020) reported that all tested varieties were susceptible to CLCuD, whether they were Bt or non-Bt cotton. Saeed *et al.* (2018) evaluated 15 different cotton genotypes against CLCuD and found that none of the genotypes were highly resistant to the disease. Only BT-980, BT-457, KIRAN, BT-666, and SLH-BT-6 exhibited a moderately resistant response. Saleem *et al.* (2017) evaluated 38 cotton genotypes and confirmed infection of CLCuMuV in 13 genotypes, CLCuKo-Bur in 24 genotypes, and 1 genotype was positive for both viruses. In another study, Soomro *et al.* (2017) evaluated 100 exotic cotton germplasms and found that 5 lines (USG15-2515, USG15-2551, USG15-2554, USG15-2555, and USG15-2556) were resistant, 39 lines were highly tolerant, 53 were tolerant, and 3 were susceptible to CLCuD in the agro-ecosystem of Sakrand, Sindh.

However, breaking the resistance in existing genotypes due to the emergence of new isolates of the begomoviruses is common (Mansoor *et al.*, 2003). Therefore, testing new and already available genotypes for resistance to the disease is crucial to avoid the distribution of susceptible hosts on a large scale. For this purpose, the present study was planned to identify resistant genotypes that can be directly used for commercial cultivation or in hybridization programs to develop new resistant varieties.

MATERIALS AND METHODS

The study was designed to identify cotton genotypes that are resistant to the stress caused by CLCuD. A total of 33 cotton genotypes, including commercial varieties and exotic genotypes such as CKC-3, CKC4, CIM-602, BF-1, BH-223, Bt.CIM-303, BT.CIM-678, Bt.CIM-789, CRIS-671, CRIS-673, CYTO-511, EYE-111, EYE-20, FH-155, FH-AM COTTON-2017, FH-Super COTTON, GH. Uhad, ICI-2424, IR-NIBGE-13, MNH-1035, MZM-7, NIAB-1011, NIAB-135, RH-670, Rohi-1, Rustam-11, Tassco-112, VH-402, CRIS-522, CRIS-613, CRIS-638, CRIS-682, and CRIS-585 were collected from various Cotton Research Institutes/Centers in Pakistan. The genotypes were evaluated against the disease in natural conditions at the experimental field of Central Cotton Research Institute Sakrand in the two growing seasons of 2018 and 2019. Each genotype was grown in three replications in a randomized complete block design (RCBD), with a row × row distance of 30 cm and plant × plant space of 75 cm. The cultural practices, such as fertilization and irrigation, were applied uniformly as required. Disease incidence (%), disease severity, and vector population were observed, with disease incidence (%) recorded by dividing the number of plants showing disease symptoms by the total number of plants used for observation and multiplying it by 100. Disease severity was calculated using the disease rating scale developed by Hassan and Chang (2017). The disease index was calculated by multiplying the disease incidence with disease severity and dividing it by the maximum severity grade of the scale (4). Observations were made at 30, 60, 90, and 120 days after sowing (DAS), and the Area Under the Disease Progress Curve (AUDPC) for CLCuD disease incidence, severity, and the index was calculated using the formula given by Campbell and Madden (1990). The data was analyzed using the statistical software Statistix-8.1, and the LSD test was applied at α=0.05.

RESULTS

Observations were taken on incidence and severity of the disease at interval of 30 DAS and then disease index was calculated for comparison between the tested genotypes. Furthermore, for summarizing the whole experiment AUDPC was calculated from the data of the disease incidence, severity and index.

Incidence of CLCuD in different genotypes under field conditions

The incidence of CLCuD was assessed in 33 different cotton genotypes under field conditions. During the initial 30 DAS, no incidence of the disease was observed in the field. In 2018, the maximum incidence of the disease was recorded in BT.CIM-678 (61.517%), followed by CRIS-585 (39.667%), CRIS-522 (33.667%), and CRIS-613 (34.00%) at 120 DAS (Table 1).

Table 1. Incidence of CLCuD on different cotton genotypes under field conditions during 2018 and 2019.

Genotype	Year	60DAS	90DAS	120DAS
CKC-3	2018	1.2000ef	5.0133d-g	6.260d-h
	2019	1.2800ef	13.297d	15.140d
CKC4	2018	0.5100ef	5.6967def	6.100d-i
	2019	0.5067ef	4.4467efg	4.443efg
CIM-602	2018	1.2733ef	3.1433d-h	3.710d-i
	2019	0.286f	0.8733fg	2.997efg
BF-1	2018	1.4700ef	6.1867def	6.187d-i
	2019	0.2900f	0.8667fg	1.723fg
BH-223	2018	3.2067ef	6.2767def	8.033de
	2019	0.6433ef	5.2967ef	5.297efg
Bt.CIM-303	2018	0.0000f	3.5700d-h	3.570d-i
	2019	0.6067ef	2.5067fg	2.507efg
BT.CIM-678	2018	26.943a	54.850a	61.517a
	2019	24.693b	51.790a	56.790a
Bt.CIM-789	2018	0.4000f	2.7800d-h	5.580d-i
	2019	0.3200f	3.1667efg	3.167efg
CRIS-671	2018	0.0000f	0.9800gh	2.370hi
	2019	0.8200ef	1.8800fg	2.423fg
CRIS-673	2018	0.5533ef	2.8400d-h	3.950d-i
	2019	0.2633f	3.2133efg	3.213efg
CYTO-511	2018	1.1800ef	6.3333de	6.337d-h
	2019	0.3100f	2.7867fg	2.787efg
EYE-111	2018	1.0633ef	6.6467de	7.723def
	2019	0.6933ef	2.9400fg	3.283efg
EYE-20	2018	0.7167ef	4.9157d-g	5.717d-i
	2019	0.3833f	1.0400fg	1.803fg

FH-155	2018	1.1667ef	2.3600e-h	2.360hi
	2019	0.3833f	2.0033fg	2.003fg
FH-AM COTTON-2017	2018	0.3567f	3.1433d-h	3.213e-i
	2019	1.1367ef	2.6533fg	3.027efg
FH-Super COTTON	2018	1.2000ef	4.3600d-h	7.013d-h
	2019	0.7633ef	1.5700fg	1.570fg
GH. Uhad	2018	0.3300	2.3533e-h	3.870d-i
	2019	0.0000f	0.8333fg	1.110g
ICI-2424	2018	0.0000f	2.7333d-h	3.930d-i
	2019	0.0000f	2.5333fg	2.533efg
IR-NIBGE-13	2018	0.0000f	1.9000fgh	3.453d-i
	2019	0.0000f	2.5700fg	2.937efg
MNH-1035	2018	0.0000f	3.8300d-h	4.937d-i
	2019	3.8400de	4.0700efg	4.070efg
MZM-7	2018	1.0100ef	6.8233d	7.417d-g
	2019	2.2000def	7.7167e	7.717e
NIAB-1011	2018	1.0667ef	2.9333d-h	4.420d-i
	2019	0.8000ef	1.2900fg	1.557fg
NIAB-135	2018	0.0000f	0.0000h	1.217i
	2019	0.0000f	1.0633fg	1.063g
RH-670	2018	0.5933ef	0.9433gh	2.530ghi
	2019	0.5933ef	1.9867fg	2.593efg
Rohi-1	2018	0.2633f	0.9500gh	2.160hi
	2019	0.0000f	0.9633fg	1.823fg
Rustam-11	2018	0.9600ef	3.9167d-h	6.027d-i
	2019	1.4500def	3.4900efg	4.913efg
Tassco-112	2018	0.2400f	3.3567d-h	3.343e-i
	2019	0.4633f	2.4067fg	2.407fg
VH-402	2018	0.2733f	1.9233fgh	2.947f-i
	2019	0.0000f	0.0000g	0.387g
CRIS-522	2018	10.667b	27.667c	33.667c
	2019	12.667c	25.000c	28.667c
CRIS-613	2018	7.6667bc	31.000bc	34.000c
	2019	10.000c	27.667c	29.667c
CRIS-638	2018	4.0000de	5.0000d-g	6.000d-i
	2019	0.3333f	4.6667ef	6.667ef
CRIS-682	2018	7.0000cd	7.0000d	8.333d
	2019	4.6667d	5.0000ef	7.667e
CRIS-585	2018	26.667a	33.667b	39.667b
	2019	28.667a	33.333b	40.000b
LSD	2018	3.5839	4.4065	4.9723
	2019	3.3480	4.6528	5.2265
P	2018	0.0000	0.0000	0.0000
	2019	0.0000	0.0000	0.0000

Similarly, during the same assessment time (120 DAS) in 2019, the maximum incidence of the disease was recorded in BT.CIM-678 (56.790%), followed by CRIS-585 (40.000%), CRIS-613 (29.667%), and CRIS-522 (28.667%) (Table 1). All remaining genotypes showed significantly lower incidence of the disease in either

2018 or 2019 (Table 1).

Statistical analysis of the Area Under the Disease Progress Curve (AUDPC) derived from the incidence % of CLCuD showed a significant difference in both years (DF=32, F=88.47, P=0.0000 and DF=32, F=225.88, P=0.0000) during 2018 and 2019, respectively. The maximum AUDPC value was determined in BT.CIM-678 (2145841.0 and 1869486.0), followed by CRIS-585 (1009075.0 and 1024330.0), CRIS-613 (570865.0 and 485925.0), and CRIS-522 (533860.0 and 457990.0) during 2018 and 2019, respectively (Figure 1a and 1b). All other varieties showed significantly lower AUDPC values during both years of observation (Figure 1a and 1b).

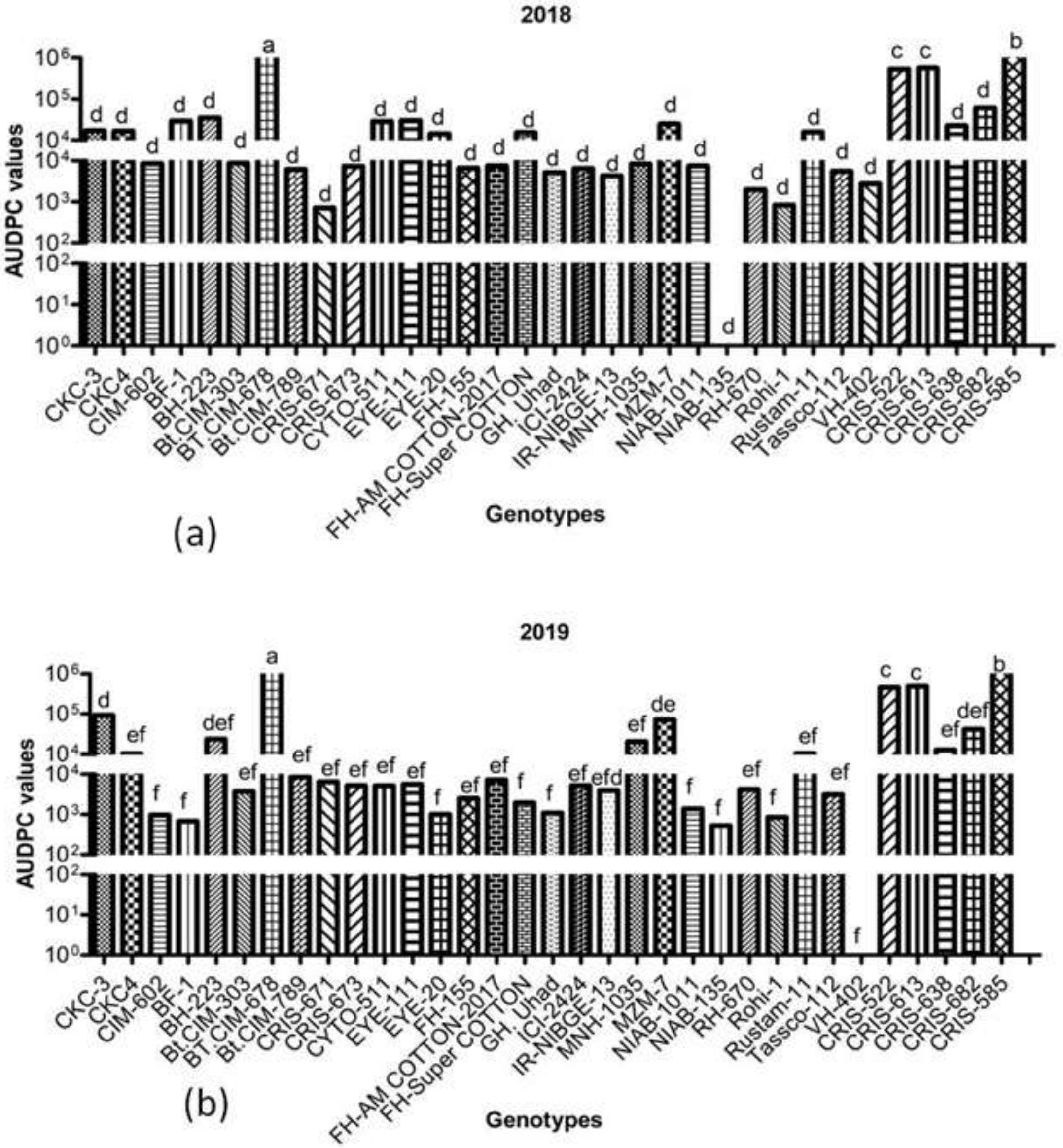


Figure 1. Area Under the Disease Progress Curve (AUDPC) calculated from incidence (%) of CLCuD on different cotton genotypes under field conditions during 2018 (a) and 2019 (b).

Severity of CLCuD in different genotypes under field conditions

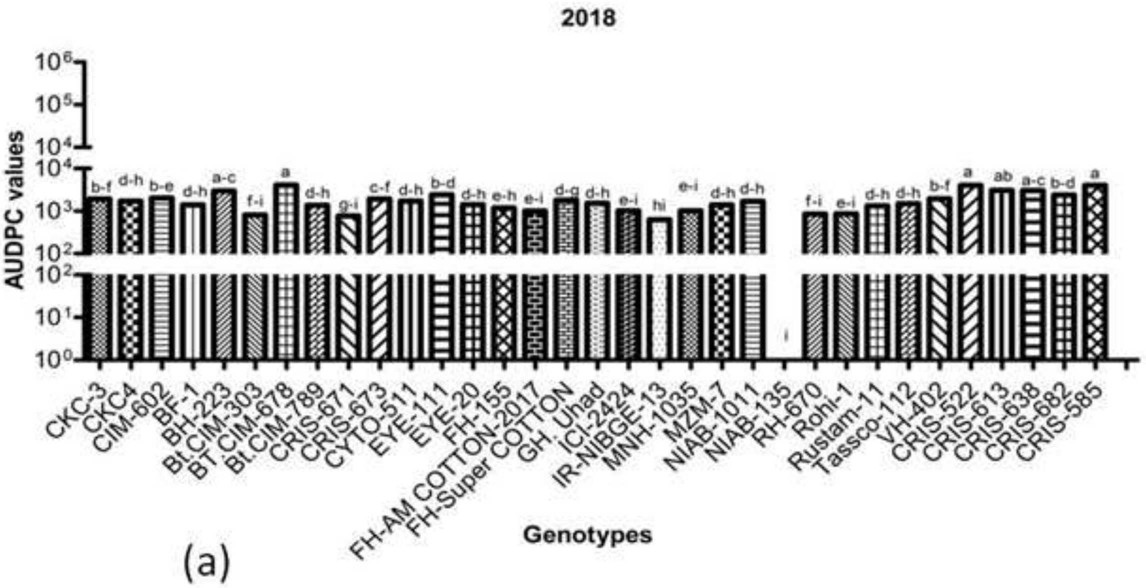
The maximum disease severity was observed in BT.CIM-678 (2.6200 and 2.1600) and CRIS-585 (2.4433 and 2.522), respectively (Table 2). The AUDPC value for disease severity was highest in BT.CIM-678 (4091.1), followed by CRIS-522 (4048.3), CRIS-585 (4003.9), CRIS-613 (3087.0), CRIS-638 (3055.7), and BH-223

(2971.8) during 2018 (Figure 2a). These values were significantly different from those of the other genotypes (DF=32, F=6.03, P=0.0000) during 2019. The remaining genotypes showed lower AUDPC values (Figure 2b), which followed a similar trend during the subsequent year of the experiment (DF=32, F=6.62, P=0.0000).

Table 2. Severity of CLCuD on different cotton genotypes under field conditions during 2018 and 2019.

Genotype	Year	60DAS	90DAS	120DAS
CKC-3	2018	0.6667bcd	1.5567a-i	2.2333a-d
	2019	1.0000abcd	1.3567b-g	2.2967ab
CKC4	2018	0.3333cd	1.5567a-i	2.4667ab
	2019	0.3333de	1.3867b-f	1.8333abc
CIM-602	2018	0.6667bcd	1.5900a-h	2.3667abc
	2019	0.3333de	0.8333f-i	1.9667abc
BF-1	2018	0.0000d	1.5833a-i	2.3467abc
	2019	0.3333de	1.0000d-i	1.3900cd
BH-223	2018	1.1333ab	1.9667a-d	2.2267a-d
	2019	0.6667cde	0.9433d-i	1.4333bcd
Bt.CIM-303	2018	0.0000d	0.9600fghi	1.5500bcd
	2019	0.3333de	1.5000bcd	1.3900cd
BT.CIM-678	2018	1.5133a	2.2233ab	2.6200a
	2019	1.0000a-d	1.2133b-h	2.1600abc
Bt.CIM-789	2018	0.3333cd	1.3333d-i	2.3067abc
	2019	0.3333de	1.0967c-i	1.5000bcd
CRIS-671	2018	0.0000d	1.1667d-i	1.7500a-d
	2019	0.3333de	1.2500b-h	1.9167abc
CRIS-673	2018	0.6667bcd	1.7233a-g	1.8867a-d
	2019	0.3333de	1.1500b-i	2.0667 abc
CYTO-511	2018	0.6667bcd	1.5033a-i	1.9533a-d
	2019	0.3333de	1.0667c-i	2.0000abc
EYE-111	2018	1.0000abc	1.6667a-g	2.3033abc
	2019	0.6667cde	1.1100b-i	1.7233abc
EYE-20	2018	0.3333cd	1.3000d-i	2.3233abc
	2019	0.6667cde	0.6667hi	1.6667abc
FH-155	2018	0.6667bcd	0.7767ij	1.6667a-d
	2019	0.3333de	1.1667b-h	1.7233abc
FH-AM COTTON-2017	2018	0.3333cd	0.9167ghi	1.3767cd
	2019	0.3333de	0.8900e-i	1.3900cd
FH-Super COTTON	2018	0.6667bcd	1.4867b-i	2.1433ad
	2019	0.6667cde	1.1667b-h	1.8333abc
GH. Uhad	2018	0.3333cd	1.2667d-i	2.0667a-d
	2019	0.0000e	0.5567ij	0.6667d
ICI-2424	2018	0.0000d	1.1333e-i	1.5200bcd
	2019	0.0000e	0.7333hi	1.3333cd
IR-NIBGE-13	2018	0.0000d	0.8333hi	1.9667a-d
	2019	0.0000e	1.1933b-h	2.0000abc
MNH-1035	2018	0.0000d	1.2767d-i	2.2367abcd
	2019	1.1333abc	1.7000b	1.9333abc
MZM-7	2018	0.3333cd	1.3900c-i	2.1667a-d
	2019	0.7100bcd	1.2400b-h	2.2900ab

NIAB-1011	2018	0.6667bcd	1.4667b-i	2.1333a-d
	2019	1.0000a-d	1.0000d-i	1.8333abc
NIAB-135	2018	0.0000d	0.0000j	1.6667a-d
	2019	0.0000e	1.0000d-i	1.5000bcd
RH-670	2018	0.3333cd	0.8333hi	1.2667d
	2019	0.6667cde	1.0667c-i	1.8900abc
Rohi-1	2018	0.3333cd	1.0000f-i	2.0000a-d
	2019	0.0000e	0.6667hi	1.5000bcd
Rustam-11	2018	0.6667bcd	1.0567fghi	2.0767a-d
	2019	1.0000a-d	0.7767ghi	2.1100abc
Tassco-112	2018	0.3333cd	1.4667b-i	2.2900abc
	2019	0.6667cde	1.0000d-i	1.8900abc
VH-402	2018	0.3333cd	1.7500a-f	2.4033ab
	2019	0.0000e	0.0000j	0.6667d
CRIS-522	2018	1.6267a	2.3100a	2.2267a-d
	2019	1.3733ab	1.5400bcd	2.0700abc
CRIS-613	2018	1.3800ab	1.8800a-e	2.2667a-d
	2019	1.2767abc	1.6267bc	1.8033abc
CRIS-638	2018	1.3867ab	1.9433abcd	2.1100a-d
	2019	0.3333de	1.5367bcd	2.1100abc
CRIS-682	2018	1.2000ab	1.6433a-g	1.9133a-d
	2019	1.2067abc	1.4667b-e	1.7900abc
CRIS-585	2018	1.6733a	2.1600abc	2.4433ab
	2019	1.5933a	2.5333a	2.5200a
LSD	2018	0.7266	0.8077	1.0169
	2019	0.6910	0.6014	0.8930
P	2018	0.0000	0.0002	0.7033
	2019	0.0000	0.0000	0.0426



(a)

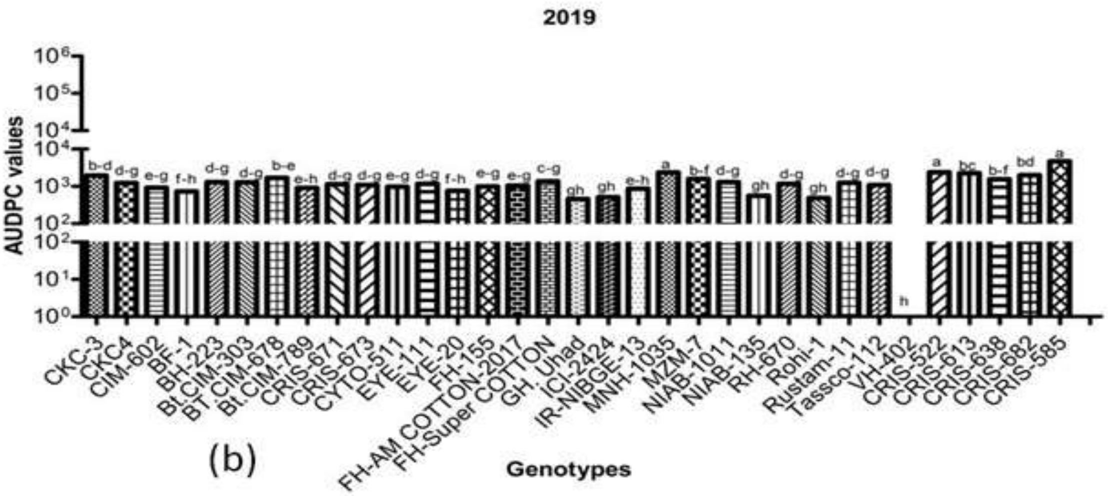


Figure 2. Area Under the Disease Progress Curve (AUDPC) calculated from severity of CLCuD on different cotton genotypes under field conditions during 2018 (a) and 2019 (b).

Index of cotton CLCuD in different genotypes under field conditions

CRIS-522, and CRIS-613 (24.13, 18.77, and 18.62, respectively) in 2018 (as shown in Table 3). However, during 2019, a significantly higher disease index (30.807) was observed in BT.CIM-678 followed by CRIS-585 (25.127), CRIS-522 (14.748), and CRIS-613 (13.403) (as presented in Table 3). The remaining genotypes showed significantly lower disease index (as illustrated in Table 3).

Table 3. Index of CLCuD on different cotton genotypes under field conditions during 2018 and 2019.

genotype	Year	60DAS	90DAS	120DAS
CKC-3	2018	0.3000e	1.9767d-g	3.53c
	2019	0.3233de	4.5233d	8.457d
CKC4	2018	0.1267e	2.2200def	3.767c
	2019	0.1267e	1.5333efg	2.023e-i
CIM-602	2018	0.3167e	1.2600d-g	2.167c
	2019	0.0733e	0.2933fg	1.187f-i
BF-1	2018	0.3667e	2.8067de	3.353c
	2019	0.0733e	0.2167fg	0.647hi
BH-223	2018	1.0133de	3.3467d	4.477c
	2019	0.1633e	1.9133ef	2.917e-h
Bt.CIM-303	2018	0.0000e	1.2467d-g	2.146c
	2019	0.3033e	0.8533fg	0.973ghi
BT.CIM-678	2018	10.536a	31.487a	38.619a
	2019	6.1667b	15.738b	30.807a
Bt.CIM-789	2018	0.1000e	0.9833efg	3.227c
	2019	0.0800e	0.9500efg	0.310hi
CRIS-671	2018	0.0000e	0.3000fg	1.063c
	2019	0.2033e	0.6733fg	1.077f-i
CRIS-673	2018	0.1400e	1.3433d-g	2.453c
	2019	0.0667e	0.9333efg	1.673f-i
CYTO-511	2018	0.2967e	2.5533de	3.383 c
	2019	0.0767e	0.7733fg	1.390fghi

eye-111	2018	0.2667e	2.6367de	4.217 c
	2019	0.1433e	0.8700fg	1.480f-i
EYE-20	2018	0.1800e	1.6267d-g	3.503c
	2019	0.1733e	0.2600fg	0.623hi
FH-155	2018	0.2933e	0.7400efg	1.323 c
	2019	0.0967e	0.5967fg	0.827ghi
FH-AM COTTON-2017	2018	0.0900e	1.0700efg	1.587c
	2019	0.2833e	0.8533fg	1.577f-i
FH-Super COTTON	2018	0.3000e	1.6467d-g	3.753c
	2019	0.1900e	0.4800fg	0.810ghi
GH. Uhad	2018	0.0833e	1.1100efg	2.147c
	2019	0.0000e	0.3467fg	0.557hi
ICI-2424	2018	0.0000e	1.1333efg	1.987c
	2019	0.0000e	0.7267fg	1.793fghi
IR-NIBGE-13	2018	0.0000e	1.0133efg	1.87c
	2019	0.0000e	0.8100fg	1.467f-i
MNH-1035	2018	0.0000e	1.2333d-g	2.867c
	2019	1.1400de	1.7967ef	2.230e-i
MZM-7	2018	0.2533e	2.3200def	4.07c
	2019	0.5333de	2.6500e	4.527e
NIAB-1011	2018	0.2667e	1.3200d-g	2.507c
	2019	0.2033e	0.3267fg	0.723ghi
NIAB-135	2018	0.0000e	0.0000g	0.68c
	2019	0.0000e	0.2667fg	0.390hi
RH-670	2018	0.1500e	0.3100fg	1.19c
	2019	0.1500e	0.5700fg	1.290f-
Rohi-1	2018	0.0667e	0.2400fg	1.123c
	2019	0.0000e	0.2400fg	0.640hi
Rustam-11	2018	0.2400e	1.0433efg	3.457c
	2019	0.3600de	0.6333fg	2.677e-i
Tassco-112	2018	0.0600e	1.2300efg	1.88c
	2019	0.1167e	0.6033fg	1.137f-i
VH-402	2018	0.0667e	0.8200efg	1.867c
	2019	0.0000e	0.0000g	0.193i
CRIS-522	2018	4.4133b	15.910c	18.773b
	2019	4.3167c	9.5883c	14.748c
CRIS-613	2018	2.7075bcd	14.687c	36.21a
	2019	3.4400c	11.333c	13.403c
CRIS-638	2018	1.4300cde	2.4700de	3.117c
	2019	0.1200e	1.7500efg	3.347efg
CRIS-682	2018	3.0900bc	2.4467de	4.457c
	2019	1.5200d	1.9767ef	3.697ef
CRIS-585	2018	10.870a	18.110b	24.13b
	2019	11.453a	21.020a	25.127b
LSD	2018	1.9750	2.115	3.3550
	2019	3.3480	4.6528	5.2265
P	2018	0.0000	0.0000	0.0000
	2019	0.0000	0.0000	0.0000

The results revealed that the highest AUDPC value was significantly recorded in BT.CIM-678 (664109.0) followed by CRIS-585 (277732.0), CRIS-613 (133917.0), and CRIS-522 (159272.0) in 2018 (as depicted in Figure 3a). Conversely, the remaining genotypes had significantly lower AUDPC values (as shown in Figure 3a). A similar trend was also observed during 2019 (as displayed in Figure 3b), which confirms the repeatability and reliability of the obtained results. The statistical analysis indicated that there was a significant difference among AUDPC values of the disease index of the tested genotypes (DF=32, F=73.25, P=0.0000 and DF=32, F=225.88, P=0.0000) in 2018 and 2019, respectively.

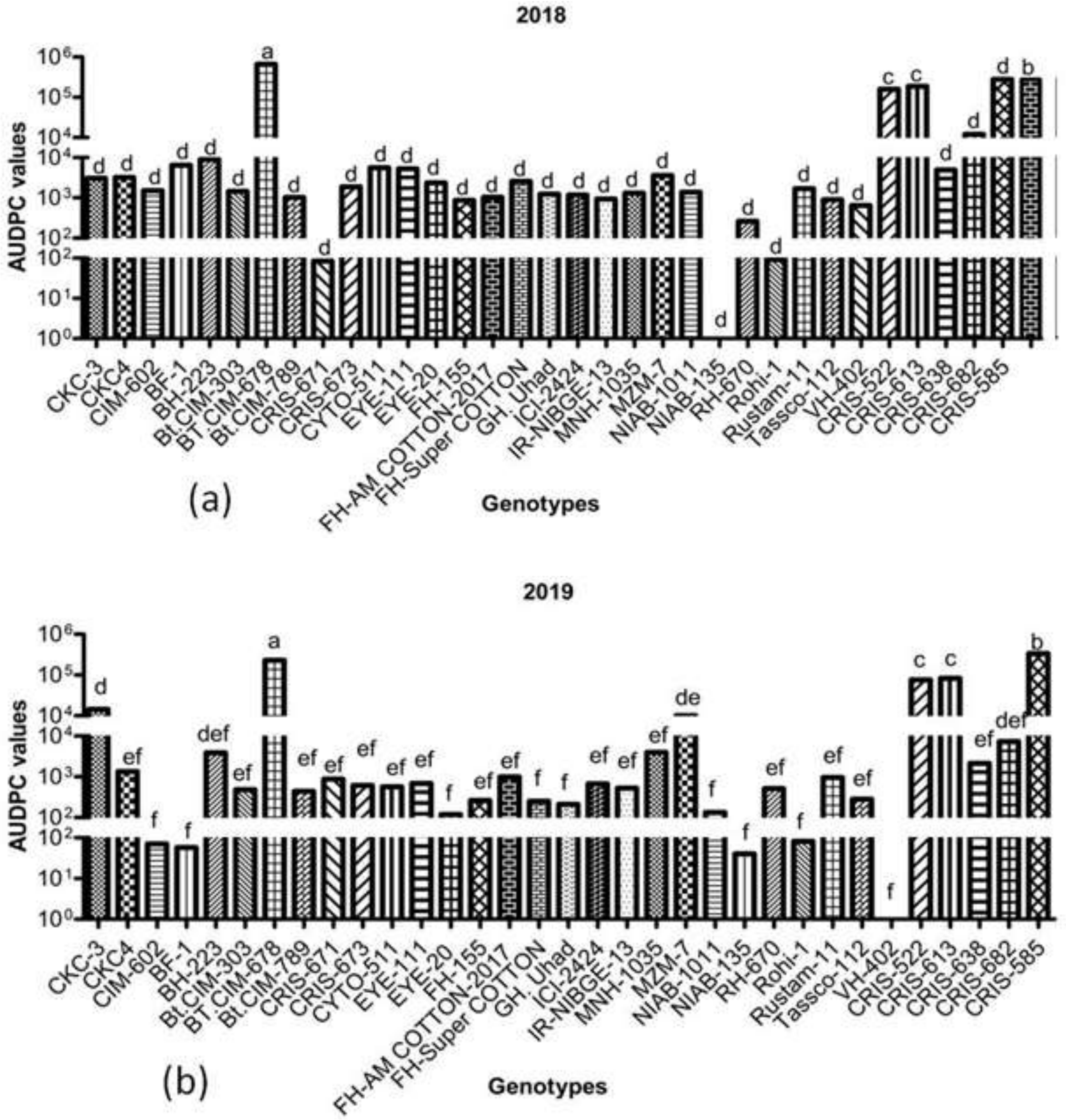


Figure 3. Area Under the Disease Progress Curve (AUDPC) calculated from index of CLCuD on different cotton genotypes under field conditions during 2018 (a) and 2019 (b).

DISCUSSION

Cotton is an economically important crop in Pakistan, serving as a source of cash for growers and producing fiber (Azhar *et al.*, 2013; Farooq *et al.*, 2014). Despite its value, the average yield of cotton is often insufficient due to the effects of various biotic and abiotic factors. Among these factors, cotton leaf curl disease (CLCuD) significantly reduces cotton yield in Pakistan, with documented reductions of up to 30% (Ashraf *et al.*, 2013; Hassan *et al.*, 2013). To address these losses, the use of resistant genotypes as a long-term strategy is highly emphasized due to their eco-friendliness, cost-effectiveness, and lack of additional required actions for growers (Rahman *et al.*, 2017). In this study, we tested 33 cotton genotypes against CLCuD in the agro-ecosystem of Sindh, Pakistan. We determined the disease incidence, severity, disease index, and AUDPC.

Significant differences in disease incidence (%) were observed among all tested genotypes at 60, 90 and 120 DAS during both 2018 and 2019. BT.CIM-678, CRIS-585, CRIS-522, and CRIS-613 showed higher incidence values at 120 DAS during both years. Conversely, all remaining genotypes displayed significantly lower disease incidence in either 2018 or 2019. These findings are consistent with previous research (Akhtar *et al.*, 2000; Ashraf and Hanif, 2010; Saleem *et al.*, 2017) and suggest that BT.CIM-678, CRIS-585, CRIS-522, and CRIS-613 genotypes are highly susceptible to CLCuD under field conditions. The consistent data obtained from both years of the study indicate that the disease is prevalent in the climatic conditions of Sindh.

A significant difference was observed in the disease severity during both years of the study at 60, 90 DAS however, there was no significant difference in disease severity among all tested genotypes at 120 DAS during 2018, but a significant difference was observed during 2019 at 120 DAS. Among all the tested genotypes, BT.CIM-678 and CRIS-585 exhibited the maximum severity score. Different cotton genotypes can display diverse severity scores to CLCuD, which is a significant disease trait that correlates with yield losses. Saghir *et al.* (2010) also reported that cotton genotypes showed different severity responses to CLCuD under field conditions. Based on our study, it can be concluded that infected cotton genotypes display varying levels of symptom expression, and our results indicate that higher expression of symptoms was observed in BT.CIM-678 and CRIS-585. Different cotton genotypes can display diverse severity scores to CLCuD, which is a significant disease trait that correlates with yield losses. Studies have reported that cotton genotypes showed different severity responses to CLCuD under field conditions. The severity of CLCuD can vary among different cotton genotypes, and infected cotton genotypes display varying levels of symptom expression (Hussain *et al.*, 2012a).

Significant differences in disease index were observed among tested genotypes in both 2018 and 2019 at each assessment point. These findings are consistent with previous research by Akhtar *et al.* (2000); Ashraf and Hanif (2010); Saghir *et al.* (2010); Hussain *et al.* (2012b); Dahab *et al.* (2013) and Humera *et al.* (2018). Therefore, it can be concluded that these genotypes are highly susceptible to CLCuD in the agro-ecological conditions of Sindh, Pakistan, based on the incidence and expression of the disease plants of cotton.

The semi-quantitative assessment of disease progression over time, known as AUDPC, differed significantly among the tested genotypes during both years of the study. BT.CIM-678, CRIS-585, CRIS-613, and CRIS-522 were highly susceptible genotypes with significantly higher AUDPC values compared to the other genotypes. These findings are consistent with previous studies conducted in the agro-ecosystem of Sindh, Pakistan, which also found that some genotypes were more resistant to CLCuD (Saghir *et al.*, 2010; Hussain *et al.*, 2012a; Dahab *et al.*, 2013; Humera *et al.*, 2018). The AUDPC can vary among different cotton genotypes and can be used to evaluate the effectiveness of different management practices and breeding programs for improving cotton crop yield in the climatic conditions of Sindh and other regions affected by CLCuD (Monga and Sain, 2021; Khan and Ilyas, 1999).

Identification of suitable genotypes with resistance or tolerance against CLCuD is important for improving cotton crop yield in Pakistan, including the climatic conditions of Sindh. The studies have assessed the genetic diversity of cotton genotypes for their CLCuD resistance/tolerance and other related agronomical traits. The results of these studies can be used for the selection of cotton genotypes with CLCuD resistance and for improving cotton crop yield (Vij *et al.*, 2022; Abro *et al.*, 2022; Javed *et al.*, 2017).

CONCLUSION

The study suggests that cotton genotypes cultivated in Pakistan have the potential to resist CLCuD attack and can be considered for large-scale cultivation and breeding programs. However, the results indicate that the cultivation of BT.CIM-678, CRIS-585, CRIS-613, and CRIS-522 should be avoided in the agro-ecosystem of Sindh due to their high susceptibility to the disease. Further testing for resistance to CLCuD under forced inoculation conditions using natural vector whiteflies or through grafting is recommended for these genotypes.

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AUTHOR'S CONTRIBUTION

All authors contributed to the study conception and design. Material preparation and data collection were performed by Nargis Shah. Analysis was performed by Jamal-U-Ddin Hajano, Manzoor Ali Abro and Abdul Mubeen Lodhi. The first draft of the manuscript was written by Nargis Shah and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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