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Assessment of genetic diversity for Cotton leaf curl virus (CLCuD), fiber quality and some morphological traits using different statistical procedures in *Gossypium hirsutum* L.

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Abstract

Cotton leaf curl virus disease (CLCuD) is one of the major factors limiting cotton production in Pakistan. For the assessment of genetic diversity one hundred cotton genotypes was evaluated for CLCuD tolerance, fiber quality, yield and some yield related traits. For evaluation of these traits cluster, principle components (PC) and correlation analysis were employed to obtain suitable parents that can be further exploited in a breeding programme. CLCuD exhibited negative and significant association with seed cotton yield (-0.317**) while with other traits it showed non-significant correlation. The ginning out turn had a significant positive correlation (0.367**) with fiber fineness but negative and significant association (-0.273**) with fiber length. For staple length only positive and significant association was found with sympodia per plant (0.210**). Plant height showed positive association (0.661**) with sympodia per plant and seed cotton yield (0.233*) while sympodia per plant showed positive and significant association with seed cotton yield (0.271**). Principal component (PC) analysis showed first 5 PCs having eigen value >1 explaining 71.3% of the total variation with plant height, sympodia per plant and seed cotton yield being the most important characters in PC1. Cluster analysis classified 100 accessions into four divergent groups. In cluster 3 and 4 included genotypes with higher tolerance of CLCuD and better fiber quality along with higher yield potential were included. Scatter plot and tree diagrams demonstrated sufficient diversity among the cotton accession for various traits and some extent of association between different clusters. The results concluded that the diversity among the genotypes could be utilized for the development of CLCuD resistant varieties with higher lint yield and germplasm conservation programs aimed at improving CLCuD tolerance with better fiber quality.

Keywords: Cotton; Cluster analysis; CLCuD; Fiber quality; Principle component analysis.
Abbreviations: CLCuD_Cotton leaf curl virus disease, GOT_Ginning out turn, SL_staple length, FF_Fiber fineness, PH_Plant height, NTFFB_Nodes to 1st fruiting branch, MPP_monopodia per plant, SPP_Sympodia per plant, NOL_Number of locules.

Introduction

Cotton is the leading fiber crop grown in more than 80 countries resulting in an annual production of about 20 million tones. In Pakistan cotton plays a key role as it is an important cash crop (Imran et al., 2011). Cotton production in Pakistan is stagnant due to a number of biotic and abiotic stresses. Among the most serious threats of the last 20 years Cotton leaf curl virus disease found to be the major factor influencing seed cotton yield (Farooq et al., 2011). Breeders all over Pakistan made efforts to develop CLCuD resistant varieties to combat this chronic disease using various breeding techniques, however, these varieties become susceptible after 2-3 years. According to Li et al. 2008 for developing elite genotypes utilization of available germplasms and addition of new germplasms resources are necessary to create sufficient genetic variation. For launching any breeding programme genetic variability is of utmost value. Variability in germplasm not only can help to increase the chances of resistance against various biotic and abiotic stresses but also can provide the chances of desirable combinations that can be utilized in future breeding endeavors (Van Esbroeck and Bowman, 1998).

The development of superior cotton genotypes by hybridization of distant parental lines has been reported (Punitha et al., 2004; Akter, 2009). To get precise information on the nature and extent of genetic variation depends upon the various techniques used for its estimation, like plant characterization based on agronomical, morphological and physiological traits (Bajracharya et al., 2006). Multivariate analysis based on Mahalonobis's D^2 statistics (MDS), principal component analysis (PCA) and principal coordinate analysis (PCoA) are mostly used approaches to evaluate the magnitude of genetic diversity among the germplasm (Brown-Guedira et al., 2000). Among these biometrical procedures the main edge of principal component analysis (PCA) is that each genotype can be assigned to only one group and it also reflects the significance of largest contributor to the total variability at each axis of differentiation (Sharma, 1998). Genetic variation for morphological traits has been estimated using principal component analysis, which lead to the recognition of phenotypic variability in cotton (Sarvanan et al., 2006; Esmail et al., 2008; Li et al., 2008). The objective of the present

Table 1. Rating scale for cotton leaf curl virus (CLCuV) symptoms (Akhtar et al., 2010).

Symptoms	Disease rating	Disease index (%)	Disease reaction
Absence of symptoms	0	0	Immune
Thickening of a few small veins or the presence of leaf enations on 10 or fewer leaves of a plant	1	0.1- 1	Highly resistant
Thickening of a small group of veins	2	1.1- 5	Resistant
Thickening of all veins but no leaf curling	3	5.1-10	Moderately resistant
Severe vein thickening and leaf curling on the top third of the plant	4	10.1 – 15	Moderately susceptible
Severe vein thickening and leaf curling on the half of the plant	5	15.1 – 20	Susceptible
Severe vein thickening, leaf curling, and stunting of the plant with reduced fruit production	6	>20	Highly susceptible

(Sum of all disease ratings/total # of plants) × 16.66.

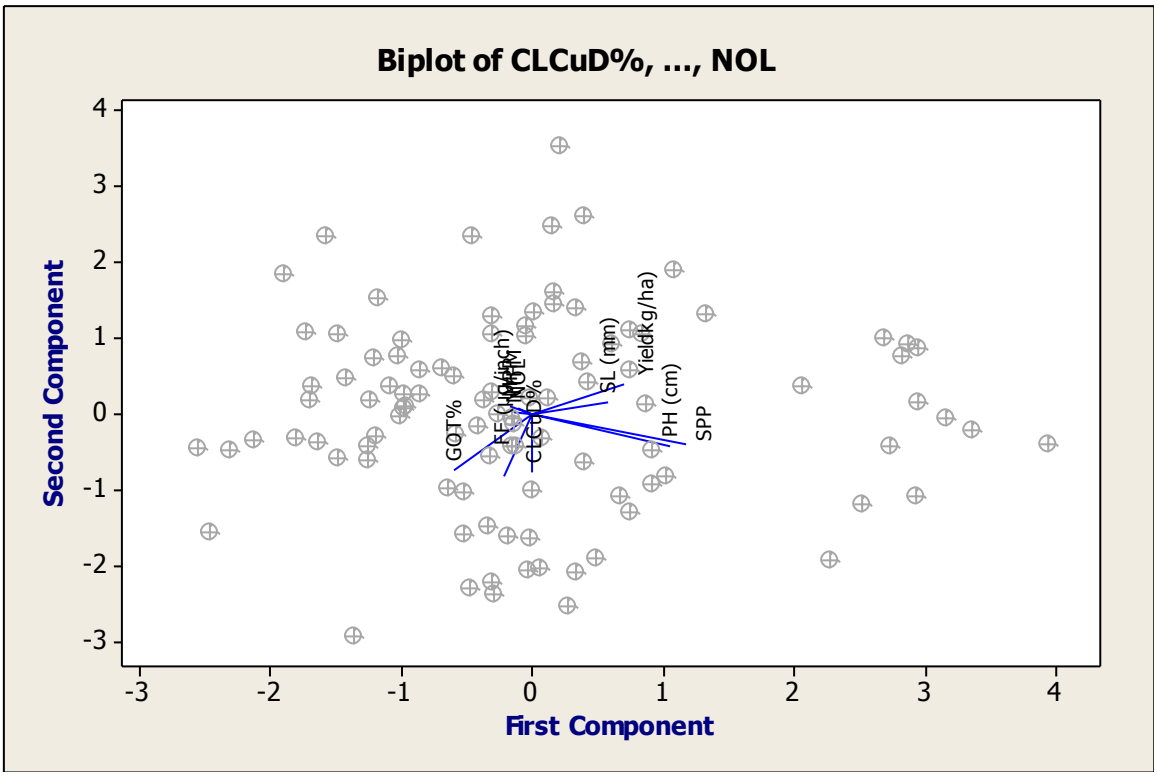


Fig 1. Biplot Between PC-1 and PC-2 showing contribution of various traits in variability of germplasm.

studies was to evaluate the genetic diversity among cotton germplasm for resistance against CLCuD , morphological and fiber traits to identify the ideal genotypes that can be exploited in breeding for aforementioned traits. Utilization of the large number of genotypes in the current experiment will yield better results especially when we consider tolerance against CLCuD.

Results

Association among various studied traits

The basic statistics of various studied traits demonstrated considerable variability among 100 cotton genotypes Table 2. Simple correlation coefficients revealed some significant associations among 10 traits (Table 3). CLCuD exhibited negative and significant association with seed cotton yield while with other traits it showed non-significant results. The GOT had a significant positive correlation with fiber fineness but negative and significant association with fiber length. For staple length only positive and significant association was found with sympodia per plant. Plant height showed positive association with sympodia per plant and seed cotton yield and sympodia per plant showed positive and significant results with seed cotton yield

Principal component analysis of various traits

In this study, out of total 11, five principal components (PCs) were extracted having Eigen value >1. These five PCs contributed 71.3% of the total variability amongst the cotton genotypes assessed for CLCuD and other yield related traits (Table 4). However, the remaining components contributed only 29.7% towards the total diversity for this set of cotton genotypes. The PC I contributed maximum towards the variability (19.5%) followed by PC II (15.3%), PC III (14.7%), PC IV (11.7%) and PC V (10.0%).The traits like sympodial branches per plant, plant height, staple length and yield kg/ha showed considerable positive factor loadings on PC I while GOT had maximum negative loadings. The 2nd PC was related to diversity among cotton genotypes due to GOT, CLCuD% and fiber fineness. The PC III was explained

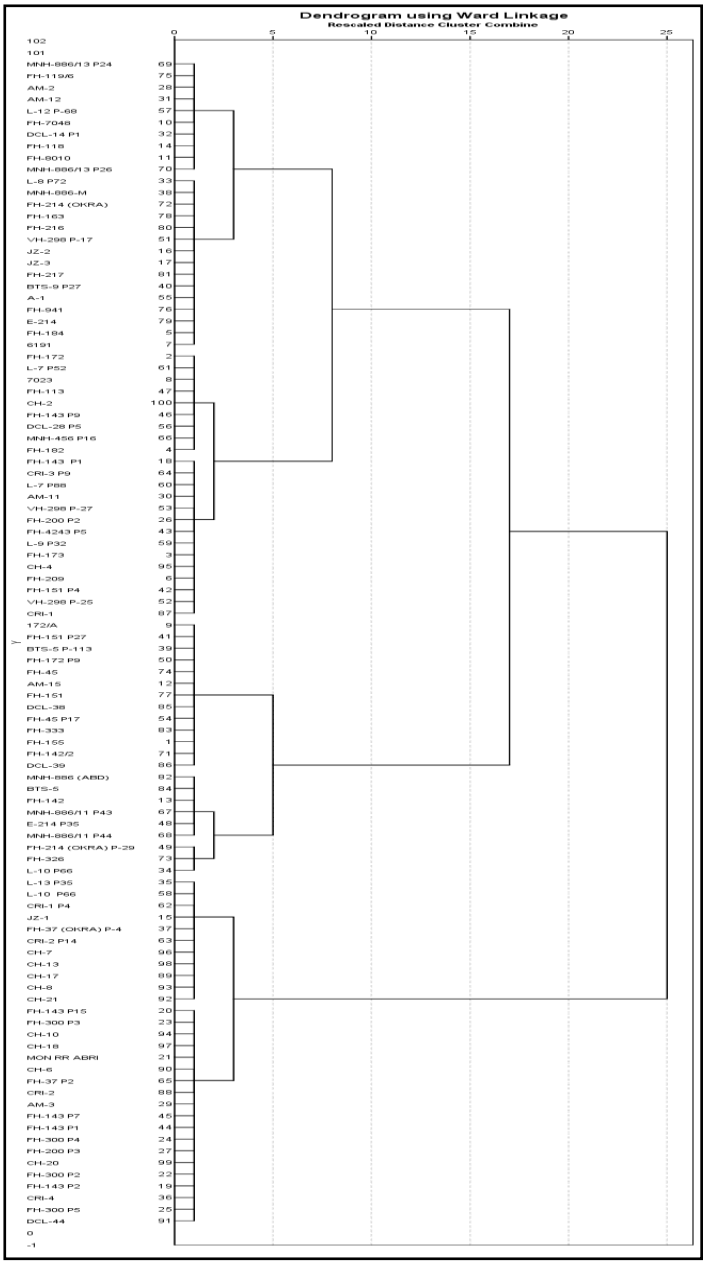
Table 2. Basic statistics for various traits of 100 cotton genotypes.

Traits	Minimum	Maximum	Mean± S.E.	Std. Deviation	Variance
CLCuD (%)	0.00	60	13.640±1.15	11.509	132.475
Yield(kg/ha)	297	4148	1430.27±77.95	779.595	607768.724
GOT (%)	33	47	40.00±0.31	3.130	9.798
SL (mm)	26.0	28.0	27.20±0.04	0.371	0.138
FF ((µg/inch)	3.0	6.0	5.10±0.051	0.512	0.262
PH (cm)	72.0	192.0	112.29±2.43	24.149	583.189
NTFFB	1	10	5.7±0.14	1.364	1.861
MPP	0	7	2.34±0.14	1.437	2.065
SPP	8	28	17.81±0.45	4.587	21.044
NOL	4	5	4.03±0.017	0.171	0.029

by variation among genotypes due to plant height, GOT, number of locules and nodes to 1stmonopodiawith their positive loadings and negative loadings exhibited by CLCuD and staple length. The PC IV was elucidated by diversity among the genotypes for yield, fiber fineness, staple length and monopodia per plant with positive loadings and number of locules exhibited negative loadings. Similarly, GOT, yield kg/ha and staple length had the considerable positive weight while nodes to 1st monopodia exhibited negative loadings on PC V. A PC biplot Figure-1 showed that variables and genotypes are super imposed on the plot as vectors. Distance of each variable with respect to PC-1 and PC-2 showed the contribution of these variables in the variation of genotypes used. The biplot showed that as a whole plant height, sympodial branches and seed cotton yield contributed maximum towards variability in germplasm.

Cluster analysis

Hundred cotton genotypes were grouped into 4 clusters based on various traits (Table 5). Cluster analysis showed that cluster 1 comprised of 31 genotypes, cluster 2 of 36 while cluster 3 had 3 and cluster 4 contained 29 genotypes (Table 6). The genotypes in cluster 1 showed reasonable values of CLCuD% and nodes to 1st fruiting branch but for other traits selection cannot be made in this cluster. Similarly, the 2nd cluster comprised of genotypes having low values of fiber fineness and CLCuD and higher values for staple length (Table 5). The members of 3rd cluster were characterized by least values of CLCuD more seed cotton yield, GOT and tall cotton genotypes. The cluster 4 is characterized by maximum GOT and number of locules, minimum nodes to 1st fruiting branch, medium in height and average in fiber fineness. The pairwise Mahalanobis distances (D^2 statistics) among four clusters of 100 cotton genotypes (Table 7) revealed that genotypes of cluster 3 showed maximum diversity against the members of cluster 4 followed by cluster 2 for most of the studied traits. The first two principal components contributed almost 34.8% towards the total variance. Not a single cluster showed obvious separation. The tree diagram showed more or less similar results comprising of two main groups A and B each of which is further sub-divided into two clusters (Figure 2).



Discussion

The information regarding association among various traits is an important part for the initiation of any breeding program as it provides an opportunity for the selection of genotypes having desirable traits simultaneously (Ali et al., 2009c). In the present set of experiment results of correlation analysis revealed some important associations among the traits studied. CLCuD showed negative association with seed cotton yield and GOT showed negative correlation while it had positive association with fiber fineness. Plant height and staple length showed positive correlation with sympodial branches which showed its positive contribution towards yield. The positive correlation among these yield contributing traits suggested that these characters are important for direct selection of high yielding genotypes. Farooq et al. 2013

Table-3. Simple correlation coefficients of various quality and morphological traits in cotton.

Traits	CLCuD (%)	GOT (%)	SL (mm)	FF (µg/inch)	PH (cm)	NTFFB	MPP	SPP
CLCuD (%)	1.000							
GOT (%)	0.141	1.000						
SL(mm)	0.146	-0.273**	1.000					
FF(µg/inch)	0.129	0.367**	-0.045	1.000				
PH(cm)	0.059	-0.072	0.009	0.073	1.000			
NTFFB	-0.012	-0.026	-0.159	0.064	0.001	1.000		
MPP	-0.024	-0.036	-0.010	0.162	-0.147	-0.009	1.000	
SPP	0.15	-0.147	0.210**	0.044	0.661**	-0.065	-0.076	1.000
NOL	-0.14	0.151	-0.099	0.044	0.013	0.074	-0.083	-0.070
Yield (kg/ha)	-0.317**	-0.112	0.112	-0.063	0.233**	0.056	0.124	0.271**

Table 4. Principle component analysis of different quality and morphological traits in cotton.

	PC I	PC II	PC III	PC IV	PC V
Eigen value	1.948	1.534	1.474	1.166	1.008
% of total variance	19.5	15.3	14.7	11.7	10.1
Cumulative variance %	19.5	34.8	49.6	61.2	71.3
Factor loadings by various traits					
Variable	PC I	PC II	PC III	PC IV	PC V
CLCuD%	0.011	-0.495	-0.411	-0.041	-0.117
Yield (kg/ha)	0.355	0.243	0.436	0.281	0.105
GOT (%)	-0.311	-0.496	0.256	-0.039	0.261
SL (mm)	0.295	0.094	-0.386	0.234	0.273
FF (µg/inch)	-0.108	-0.536	0.203	0.388	0.031
PH (cm)	0.542	-0.288	0.187	-0.147	-0.100
NTFFB	-0.069	0.011	0.291	-0.079	-0.808
MPP	-0.093	0.053	0.031	0.808	-0.135
SPP	0.606	-0.259	0.025	0.001	-0.043
NOL	-0.054	0.035	0.513	-0.081	0.385

Table-5. Cluster analysis of various traits in cotton.

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4
CLCuD (%)	1.0	2.0	0.0	2.0
Yield(kg/ha)	297	1506	4148	2820
GOT (%)	34	37	39	42
SL (mm)	27.1	27.3	27.0	27.2
FF ((µg/inch)	4.8	3.0	5.1	4.6
PH (cm)	125	82	142	117
NTFFB	5	7	7	5
MPP	3	1	1	2
SPP	21	18	25	16
NOL	4	4	4	5

Table 6. Cluster membership of various genotypes.

Cluster-1	31	JZ-1, FH-143 P2, FH-143 P15, MON RR ABRI, FH-300 P2, FH-300 P3, FH-300 P4, FH-300 P5, FH-200 P3, AM-3, L-13 P35, CRI-4, FH-37 (OKRA) P-4, FH-143 P1, FH-143 P7, L-10 P66,CRI-1 P4, CRI-2 P14, FH-37 P2, CRI-1, CRI-2, CH-17, CH-6, DCL-44, CH-21, CH-8, CH-10, CH-7, CH-18, CH-13, CH-20
Cluster-2	36	FH-172, FH-173, FH-182, FH-209, 7023, FH-7048, FH-8010, FH-118, FH-143 P1, FH-200 P2,AM-2, AM-11, AM-12, DCL-14 P1, FH-151 P4, FH-4243 P5, FH-143 P9, FH-113, VH-298 P-17, VH-298 P-25, VH-298 P-27, DCL-28 P5, L-12 P-68, L-9 P32, L-7 P88, L-7 P52, CRI-3 P9, MNH-456 P16, MNH-886/13 P24, MNH-886/13 P26, FH-214 (OKRA), FH-119/6, FH-163, FH-216, CH-4, CH-2
Cluster-3	3	L-10 P66, FH-214 (OKRA) P-29, FH-326
Cluster-4	29	FH-155, FH-184, 6191, 172/A, AM-15, FH-142, JZ-2, JZ-3, L-8 P72, MNH-886-M, BTS-5 P-113, BTS-9 P27, FH-151 P27, E-214 P35, FH-172 P9, FH-45 P17, A-One,MNH-886/11 P43, MNH-886/11 P44, FH-45, FH-941, FH-151, E-214, FH-217, MNH-886 (ABD), FH-333, BTS-5, DCL-38, DCL-39, FH-142/2

Table 7. D^2 statistics among different clusters.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	0.000			
Cluster 2	711.555	0.000		
Cluster 3	2989.054	2277.518	0.000	
Cluster 4	1604.169	892.655	1384.896	0.000

reported positive correlation of yield with yield contributing traits and also found negative association between seed cotton yield and CLCuD. The maintenance and exploitation of genetic resources could be made by partitioning the total variance into its components. It also provides a chance for utilization of appropriate germplasm in crop improvement for particular plant traits (Sneath and Sokal, 1973; Pecetti et al., 1996). The principal components analysis is a powerful tool to obtain parental lines for successful breeding program (Akter et al., 2009). In this experiment, the PC analysis partitioned the total variance into 5 PCs contributing maximum to the total diversity among the genotypes. Chozin (2007) and Mujaju and Chakuya (2008) reported important contribution of first PCs in total variability while studying different traits. In our experiment, 1st PC was mainly due to variations in sympodial branches per plant, plant height, GOT and seed cotton yield. These results are in agreement with the results of association analysis which showed positive association among these traits. The 2nd PC was related to diversity among cotton genotypes due to CLCuD, GOT and fiber fineness. The PC III was explained by variation among genotypes due to CLCuD, GOT and seed cotton yield. Similarly, PC IV was explicated by variation in monopodia per plant and fiber fineness with their considerable positive factor loadings. PC analysis ultimately confirmed the amount of variation for the traits among the material studied which could be utilized in designing a breeding program aimed at improving CLCuD tolerance, fiber quality and ultimately seed cotton yield as it is generally assumed that maximum variation yields maximum heterotic effects. Malik et al. 2011 and Ashokkumar and Ravikesavan 2011 reported that the presence of sufficient amount of variability in colored cotton genotypes offer ample scope for characterization of colored cotton genotypes.

The cluster analysis also corroborates sufficient diversity for this group of genotypes at various growth stages. The genotypes in cluster 3 & 4 comprised of genotypes with less attack of CLCuD and more seed cotton yield having better fiber quality traits. Though cluster 1 and 2 comprised of some genotypes having better tolerance against CLCuD% but all have poor yield. Both cluster 3 and 4 could be utilized in breeding programmes due to availability of sufficient amount of useful variability regarding yield contributing traits and CLCuD. The pair wise Mahalanobis distances (D^2 statistics) among four clusters revealed that genotypes of cluster 3 showing maximum diversity against the members of other clusters for most of the studied traits so could be exploited mainly to obtain transgressive segregates. The scatter plot showed association between different clusters which could be because a large number of genotypes showing variation in adaptation or some sort of similarity between them. However, cluster analysis brings together those genotypes showing considerable tolerance against CLCuD tolerance at one place and on the basis of yield related traits at other place. Amurrio et al. (1995) and Rabbani et al. (1998) reported lack of relationship between various clusters based on agronomic traits and origins of genotype in peas (*Pisumsativum*) and mustard (*Brassica juncea*) respectively. The tree diagram comprised of group of genotypes showing considerable tolerance against CLCuD and possessing better yield potential. The occurrence of this wide variation between the clusters is of great genetic value in providing genotypes aimed at cotton selection for adaptation to CLCuD affected areas. Similar kind of results related to germplasms grouping has been reported by Ayana and Bekele 1998 and Grenier et al. 2001.

Materials and methods

Plant Material and site characteristics

Hundred genotypes were evaluated during these studies. These studies were carried out during the cropping seasons 2012-13 (Table-6). The sowing was done on of May 25, 2012. The experiment was carried out at Cotton Research Institute, Faisalabad, Punjab, Pakistan. The germplasm utilized for the studies were homogeneous and randomly selected from F_6 and F_7.

Experimental design, plot Size and cultural practices

For each entry, plot size measured 4.572 m × 1.524 m, comprising 2 rows set 75 cm apart. Distance between plants within rows was 30 cm. Normal agronomic and cultural practices (irrigation, weeding, hoeing, and fertilizer applications) were adopted as and when required.

Measurement of the studied traits

For measuring the traits 10 representative, undamaged plants were selected in each line and marked for identification. Nodes to 1st fruiting branch counted from zero node (cotyledonary node) to the node at which first flower was appeared. Data on plant height in centimeters were recorded from the base of the plant to the tip of the plant. Data on monopodia and sympodia were taken by counting the number of vegetative and fruiting branches. Seed cotton was picked in two pickings. 1st pick was done after 120 days and 2nd pick was done at maturity and recorded as Kg / plot and extrapolated in Kg / hectare. The harvest was weighed with the help of electrical balance and final averages were made from each tagged plant. Cleaned and dry samples of seed cotton were weighed and then ginned separately with single roller electric ginning machine. The lint obtained from each sample was weighed and ginning out turn % was calculated by the following formula

Ginning outturn (%) = Weight of lint / Weight of seed cotton × 100

Fiber characteristics such as staple length, fiber fineness of each guarded plant were measured by using spin lab HVI-900. This computerized instrument provides us a true profile of raw fiber. It measures the most important characters such as staple length (mm) and fiber fineness (μg/inch) within a quick period of time according to international trading standards.

CLCuD Disease Incidence (%) Methodology

CLCuV disease incidence (%) and reaction of the cultivars was determined using the disease scale (Table 1) described by Akhtar et al. 2010 and Farooq et al. 2011. Then %age of CLCuD incidence was calculated by using the following formula;

CLCuV disease incidence (%) = Sum of all disease ratings/total number of plants ×16.16

Statistical Analysis

The average was subjected to basic statistics, correlation analysis, cluster analysis and principal component analysis (PCA) using statistical software packages of SPSS version 19 and STATISTICA version 5.0 (Sneath and Sokal, 1973). Cluster analysis was performed using K-means clustering

while tree diagram based on elucidation distances was developed by Ward's method. The D^2 statistics was calculated according to Mahalnobis (1936) and Rao (1952). First two principal components were plotted against each other to find out the patterns of variability among genotypes and association between different clusters using SPSS version 19.

Conclusion

PC analysis, Cluster analysis and correlation coefficient in this present set of experiment provided facilitation in the classification of genotypes and identification of subset of genotypes having tolerance to CLCuD, better fiber quality and possessing good yield potential. Various useful correlations and aforementioned information extracted from cluster and PC analysis will be helpful in designing breeding programmes to obtain high yielding genotypes possessing high degree of tolerance against CLCuD and better fiber quality.

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