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GENETIC DIVERSITY IN UPLAND COTTON FOR COTTON LEAF CURL VIRUS DISEASE, EARLINESS AND FIBER QUALITY

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ABSTRACT:- In Pakistan during last two decades the major factor limiting cotton production is cotton leaf curl virus disease (CLCuD). For estimation of genetic diversity regarding CLCuD tolerance, fiber quality and some yield contributing traits, 101 cotton genotypes imported from USA were evaluated. Different statistical procedures like cluster, principle components (PC) and correlation analysis were employed to identify the suitable genotypes that can be further exploited in breeding programme. Significant associations were found between yield contributing trait, boll weight and fiber related trait, staple length. Earliness related traits, like days taken to 1st square and days taken to 1st flower had positive correlation with each other and both these traits also showed their positive association with ginning out turn. The negative significant correlation of CLCuD was obtained with monopodial branches, sympodial branches and plant height. Principal component (PC) analysis showed first five PCs having eigen value >1 explaining 67.8% of the total variation with days to 1st square and flowering along with plant height and sympodia plant^{-1} which were being the most important characters in PC1. Cluster analysis classified 101 accessions into five divergent groups. The genotypes in cluster 1 only showed reasonable values for days to 1st square and flower, sympodia per plant, ginning out turn, staple length and fiber fineness and the genotypes in cluster 5 showed promising values for the traits like cotton leaf curl virus, ginning out turn and fiber fineness. The genotypes in cluster 1 and 5 may be combined to obtain desirable traits related to earliness and better disease tolerance. Scatter plot and tree diagrams demonstrated sufficient diversity among the cotton accessions for various traits and some extent of association between various clusters. It is concluded that diversity among the genotypes could be utilized for the development of CLCuD resistant lines with increased seed cotton and lint yields with desirable fiber quality.

Key Words: Cotton; Cotton Leaf Curl Virus Disease; Correlation; Earliness; Ginning Out Turn; Cluster Analysis; Yield Components; Pakistan.

INTRODUCTION

Cotton plays a pivotal role in boosting our national economy being the chief source of earning foreign exchange. Economy of Pakistan is largely reliant on production of upland cotton, with 1815 textile and

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ginning units supported by millions of laborers, farmers and traders, earning livelihoods directly or indirectly from this crop (Khan, 2013; Khan and Hassan, 2011). It is also termed as white gold due to its significance as cash and industrial crop.

In Pakistan the cotton production is stagnant for last two decades due to many biotic and abiotic stresses. Among these threats, the most damaging factor is cotton leaf curl virus which is responsible for huge losses in cotton production (Farooq et al., 2011). Breeders all over Pakistan made efforts to develop CLCuD resistant varieties to combat this disease using various breeding techniques, however, these varieties become susceptible after 2-3 years. According to Li et al. (2008) for obtaining superior genotypes proper exploitation of available germplasm in the form of hybridization and addition of new germplasm are necessary to create sufficient genetic variation. Variability in germplasm not only increase the chances of multiple resistance against biotic and abiotic stresses but also yield desirable combinations that can be utilized in future breeding prog-rammes (Van Esbroeck and Bowman, 1998). Different breeding procedures like introduction of exotic germ-plasm, hybridization and polyploidy can be used to obtain the desired genetic variability and the genotypes with diverse segregating populations (Esmail et al., 2008).

The past studies (Punitha and Raveendran, 2004; Akter et al. 2009) reported the development of cotton genotypes by hybridizing the distant parents. The precise information on the nature and magnitude of genotypic variation depends upon the various procedures exploited for its estimation like characterization based on agronomical, morphological and physiological traits (Bajracharya et al., 2006). Multivariate analysis based on Mahalonobis's D2 statistics (MDS), principal component analysis (PCA) and principal coordinate analysis (PCoA) are most exploited approaches to estimate the extent of genetic diversity among the germplasm (Brown-Guedira et al., 2000). Among these biometrical techniques, the main advantage of PCA is that each genotype can be assigned to only one group and it also reflects the worth of largest contributor to the total variability at each axis of differentiation (Sharma, 1998). Genetic variation for morphological traits has been estimated using PCA, which showed the way to the identification of phenotypic variability in cotton (Saravanan et al., 2006; Esmail et al., 2008; Li et al., 2008).

The objective of the present study was to evaluate and identify the cotton germplasm with resistance to CLCuD, and desirable morphological and fiber quality traits.

MATERIALS AND METHOD

Plant Material and Site Characteristics

One hundred and one cotton genotypes imported from USA were evaluated during the crop season 2012-13. The experiment was carried out at Cotton Research Institute, Faisalabad, Punjab, Pakistan, that lies at an altitude of 184m at 31° 21' 52″ N 72° 59' 40″ E with average rainfall of 300mm. The sowing was done on June 19, 2012 which is about 35 days later than the optimum sowing time as during late sown conditions the crop naturally receive

Table 1. Rating scale for cotton leaf curl virus disease (CLCuD) symptoms

Symptom	Disease rating	Disease index (%)	Disease reaction immune
No symptom	0	0	Immune
Thickening of only secondary and tertiary veins	1	0.1-10	Highly tolerant
Thickening of tertiary veins, secondary and primary veins	2	10-30	Tolerant
Vein thickening, leaf curl or venation or both	3	30-50	Susceptible
Stunting alone with vein thickening, leaf curl or venation	4	>50	Highly susceptible

Table 2. Basic statistics for various traits of 101 cotton genotypes

Trait	Minimum	Maximum	Mean± S.E.	Std. Deviation	Variance
Days to 1st square	30	58	39.15 ± 0.74	7.51	56.40
Days to 1st flower	47	74	57.08 ± 0.68	6.94	48.14
Plant height	38	90	56.92 ± 0.84	8.53	72.76
Nodes to 1st monopodia	5	11	6.87 ± 0.12	1.19	1.41
Monopodia plant^{-1}	0	6	2.58 ± 0.13	1.35	1.83
Sympodia plant^{-1}	8	18	12.76 ± 0.20	2.04	4.15
CLCuD%	5	100	68.25 ± 1.58	16.03	256.96
No. of locules	4	5	85.28 ± 0.29	2.45	6.00
Boll weight (g)	1.3	5.1	2.82 ± 0.06	0.65	0.42
Ginning out turn (%)	28	44	35.96 ± 0.38	3.80	14.41
Staple length (mm)	26	30	27.60 ± 0.05	0.54	0.29
Fibre fineness (µg inch^{-1})	4.0	6.2	5.09 ± 0.05	0.52	0.27

CLCuD = Cotton leaf curl virus disease

cotton leaf curl virus symptoms due to more attack of insect pests especially whitefly and none of the genotypes were sprayed against white fly. The germplasm utilized have sufficient amount of genetic variability regarding CLCuD, fiber quality and earliness related traits. The

information regarding the genotypes can be obtained by visiting the web-site (http://www.ars-grin.gov/npgs/).

Experimental Design

For each entry, plot size was 6.096 m × 1.524 m, comprising two rows set 75 cm apart, and 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 Traits

For measuring the traits, the 10 representative and undamaged plants were selected in each line and marked for identification. Data were collected for nodes to 1st fruiting branch counted from zero node (cotyledonary node) to the node at which first flower had appeared, number of days to 1st square and 1st flower, plant height, monopodia and sympodia per plant, boll weight, number of locules and ginning out turn (GOT). For GOT, the 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.

Fiber quality characteristics such as staple length, fiber fineness of each guarded plant were measured by using spin lab HVI-900. It measures the most important characters such as staple length (mm) and fiber fineness (µg inch^{-1}) according to international trading standards.

CLCuD Incidence (%)

Cotton leaf curl virus disease incidence (%) and reaction of the cultivars was determined by using disease scale (Table 1) modified from the scale described by Akhtar et al. (2010) and Farooq et al. (2011). Then percentage of CLCuD incidence was calculated by using the following formula.

CLCuD incidence (%) = Sum of all disease ratings/total number of plants ×25

Statistical Analysis

The average data of all the traits were subjected to basic statistics, correlation analysis, cluster analysis and 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. The D2 statistics was calculated according to Mahalanobis (1936) and Rao (1952).

RESULTS AND DISCUSSION

Correlation Studies

The basic statistics of various studied traits demonstrated considerable variability among 101 genotypes (Table 2). Simple correlation coefficients revealed some significant associations among 12 studied traits (Table 3). The association among various traits is an important aspect for the initiation of any breeding programme as it offers chances for the selection of genotypes having desirable traits simultaneously (Ali et al., 2009). Significant associations were found between yield contri-buting trait, boll weight and fiber related trait, staple length. Earliness related traits, like days taken to 1st square and days taken to 1st flower had positive correlation with each other and both these traits also showed

Table 3. Simple correlation coefficients of CLCuD, earliness and quality traits in some genotypes of cotton

Traits	BW	CLCV	DFS	DFF	GOT%	FF	MP	NOL	NTFFB	PH	SL
CLCV	0.019										
DFS	-0.178	-0.210									
DFF	-0.132	-0.174	0.921**								
GOT	-0.049	-0.127	0.208**	0.197*							
FF	0.095	0.017	-0.096	-0.098	0.135						
MP	0.064	-0.256**	0.072	0.033	0.061	-0.034					
NOL	0.129	0.178	-0.132	-0.102	0.052	0.167	0.069				
NTFFB	0.027	0.032	-0.042	0.007	-0.041	-0.169	-0.073	0.031			
PH	0.072	-0.219**	-0.159	-0.222*	0.005	-0.108	0.426**	-0.081	0.008		
SL	0.190*	0.105	-0.001	0.023	-0.275**	-0.257**	-0.081	0.089	-0.059	-0.038	
SB	0.071	-0.380**	-0.126	-0.171	0.012	-0.200*	0.149	-0.178	-0.101	0.54**	-0.075

GOT=Ginning out turn, FF= Fiber fineness, DFS= Days taken to 1st square, DFF= Days taken to 1st flower, MP= Monopodia per plant, NOL= Number of Locules, NTFFB= Nodes to 1st fruiting branch, PH=Plant height, SL= Staple length, SB= Sympodial branches, MP= Monopodia per plant, BW= Boll weight
** and ** = Significant at 1% and 5% level, respectively.*

their association with ginning out turn. Similar kind of association was found in earlier studies (Farooq et al., 2013), who concluded positive association between days to 1st square and flower under virus intensive conditions. However, days to 1st flower showed negative association with plant height. The significant negative correlation of CLCuD was obtained with monopodial branches, sympodial branches and plant height. GOT exhibited significant negative association with staple length. Likewise, fiber fineness showed negative correlation with staple length and sympodial branches. Farooq et al. (2013) reported positive correlation of yield with yield contributing traits. These associations may be considered while selecting parents for future hybridization programmes especially under late sowing conditions.

Principle Component Analysis (PCA)

The preservation and utilization of genetic resources could be made by partitioning the total variance into its components. It also provides a chance for exploitation of suitable germplasm in crop improvement for particular plant traits (Pecetti et al., 1996). PCA is an influential tool to obtain parental

Table 4. Principle component analysis of CLCuD, earliness and quality traits in some genotypes of cotton

Variable	PC I	PC II	PC III	PC IV	PC V
Eigen value	2.250	2.110	1.490	1.220	1.060
Total variance (%)	18.700	17.600	12.400	10.230	8.900
Cumulative variance (%)	18.700	36.300	48.700	58.900	67.800
Days to 1st square	-0.600	-0.170	0.090	0.180	0.010
Days to 1st flower	-0.600	-0.120	0.110	0.170	-0.030
Plant height (cm)	0.303	-0.470	0.022	0.085	-0.122
Nodes to 1st fruiting branch	0.006	0.068	0.165	-0.171	-0.858
Monopodia per plant	0.066	-0.383	-0.117	0.399	-0.171
Sympodia per plant	0.248	-0.489	0.087	-0.107	0.122
No. of locules	0.095	0.232	-0.217	0.512	-0.281
Boll weight (g)	0.215	0.038	0.032	0.536	-0.031
Ginning out turn (%)	-0.212	-0.146	-0.458	0.023	-0.171
Staple length (mm)	0.050	0.142	0.548	0.413	0.206
Fiber fineness (μg inch^{-1})	0.023	0.156	-0.608	0.119	0.218
CLCuD (%)	0.087	0.474	0.053	-0.027	-0.061

lines for successful breeding programmes (Akter et al., 2009; Nazir et al., 2013).

In this study out of total 12, five principal components (PCs) were extracted having eigen value >1. These five PCs contributed 67.8% of the total variability amongst the genotypes assessed for CLCuD, earliness and fiber related traits (Table 4). However, the remaining components contributed only 32.2% towards the total diversity. The PC I contributed maximum towards the variability (18.7%) followed by PC II (17.6%), PC III (12.4%), PC IV (10.2%) and PC V (8.9%). Mujaju and Chakuya (2008) and Nazir et al. (2013) reported important contribution of first PCs in total variability while studying different traits.

The traits like days to 1st square and flower showed considerable negative factor loadings on PC I while plant height, sympodia per plant and boll weight had maximum positive loadings. Maximum positive loading on PC II was shown by CLCuD while maximum negative loadings were exhibited by traits like plant height, sympodia per plant. The negative loadings on PC I and PC II were shown by staple length on both components while the traits like CLCuD and days to 1st boll opening showed negative loadings only on PC II. The PC III was elucidated by diversity among the genotypes for staple length with positive and fiber fineness and GOT with maximum negative loadings. Boll weight, monopodia per plant and staple length showed considerable positive loadings on PC IV and in PC V maximum negative loadings were shown by nodes to 1st monopodia. PC analysis confirmed the extent of variation for the traits among the material studied which could be utilized in designing a breeding programme aimed at improving CLCuD tolerance, fiber quality and ultimately seed cotton yield as it is generally assumed that maximum variation yield maximum heterotic effects (Nazir et al., 2013). Malik et al. (2011) and Ashokkumar and Ravikesavan (2011) mentioned the presence of ample amount of variation in colored cotton genotypes offer huge scope for characterization of colored cotton genotypes. The results are in agreement with the findings of Nazir et al. (2013) who found the contribution of first two principle components while studying different cross combinations.

Cluster Analysis

This is another useful statistical procedure to obtain genotypes from various clusters having desirable traits. One hundred genotypes were grouped into 5 clusters based on various traits (Table 5). Cluster analysis showed that cluster 1 comprised, 27; cluster 2, 22; cluster 3, 9; cluster 4, 13; and cluster 5, 30 genotypes (Table 6). The genotypes in cluster 1 only showed reasonable values of days to 1st square and flower, sympodia per plant, GOT, staple length and fiber fineness but for other traits selection cannot be made. Similarly, cluster 2 comprised genotypes having only promising values for GOT (Table 5). The members of cluster 3 were characterized by better values of boll weight and staple length. The members of cluster 4 showed better results with respect to earliness related traits like days to 1st square and flower along with best boll weight and staple length but with respect to CLCuD this

cluster showed more susceptible genotypes. The genotypes in cluster 5 showed very good results with respect to CLCuD, GOT and fiber fineness. The genotypes in cluster 1 and 5 may be combined to obtain desirable traits related to earliness and better disease tolerance. 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 (*Pisum sativum*) and mustard (*Bra-ssica juncea*) respectively. Similarly wide variations in clusters have been reported by Nazir et al. (2013). The occurrence of wide variation between the clusters is of great genetic value in providing genotypes aimed at cotton selection for adaptation to CLCuD hit areas. Similar kind of results associated to germplasm grouping has been reported (Ayana and Bekele, 1998; Grenier et al., 2001). The pairwise Mahalanobis distances (D2 statistics) among five clusters of 100 cotton genotypes (Table 7) revealed that genotypes of cluster 2 and 5 showed maximum diversity against the members of other clusters. The first two principal components contributed almost 36.7% towards the total variance. Not a single cluster showed obvious separation.

It is thus concluded that correlation, cluster and PC analysis facilitated the detection of genotypes having tolerance to CLCuD, better fiber quality and possessing earliness. Various useful correlations and above mentioned information extracted from cluster and PC analysis will be helpful in designing breeding programmes to obtain high yielding genotypes possessing high degree of CLCuD tolerance and better fiber

Table 5. Cluster analysis of CLCuD, earliness and quality traits in various genotypes of cotton

	Cluster				
Variable	1	2	3	4	5
Days to 1st square	36.00	55.00	56.00	32.00	58.00
Days to 1st flower	51.00	71.00	71.00	50.00	73.00
Plant height (cm)	61.00	43.00	48.00	65.00	68.00
Nodes to 1st fruiting branch	6.00	7.00	7.00	6.00	7.00
Monopodia per plant	4.00	0.00	5.00	3.00	4.00
Sympodia per plant	16.00	10.00	14.00	13.00	14.00
No. of Locules	4.00	4.00	4.00	4.00	4.00
Boll weight (g)	2.50	2.10	2.60	3.10	2.50
Ginning out turn (%)	40.00	39.00	36.00	36.00	43.00
Staple length (mm)	27.30	27.00	27.60	28.30	27.40
Fiber Fineness (µg/inch)	4.80	5.60	5.10	5.00	4.90
CLCuD (%)	29.00	100.00	55.00	72.00	7.00

Table 6. Cluster membership of various genotypes

Cluster 1	27	DES 56, DES 119 Var1, DES 24, Halima c2, DP 16 ne, Bob Shaw 54-A, Bob Shaw 54-B, SJ-1, Riata, Dolcot 277, SG 125, SG 501, JACO 6078 (JAJO), Tash Kent I, Hancock, B 3080, DPL 5690, Yugo 8, Coker 413-8913, FM 963, FM 958, STV 213 2007 Plot 3005, JAJO 8083 (JACO), S9 2008 self, N77, STV GL, HPR ne03
Cluster 2	22	DES 56 ne, Atlas 67, Del Cerro, Gumbo 500, DP 65-1, DP 65-11 Okra, Northern Star, Raider, Lone Star cluster, Lone Star, GREGG 65 gl2 gl3, Party stoole, DPL 26, la 887, AZ 64, Aub 56, Meredith 84-1-29, STV & A 125, L2 2007 Plot 2511, DP 383, PM H 1220 (1990), JACO GL
Cluster 3	9	FTA 263-20, ARK YUGO JS source, TM-1 g.H. 2004 4016-1, NM 970513 2007, DES 119H, PHY 72, Acala 1517, GVS 2 STV 114, GVS 5 STV gl
Cluster 4	13	FTA 26320, DP 16 Hairyb, Acala S-2, Dix King II ne, FM 1006, Lankart 65, SG 105, FOX 4, Ark Yugo, FM 832 ne, Stampf Halima 165, Mac7, DNWC1324
Cluster 5	30	DES 24 ne, Atlas 66, NC 7212, STV 42, Dp 65-11 Norm, Dp65-11 Sub, DPL SML ne, DP 16 Hairya, DP 16 Var, COKer 310, Jermans 510, McNair 235, Dix King, Aub-634-101 RNR, GREGG 65, HQ-75-Norm, Coker 312, DELTA, PEARL, GA KING, Sphinx, CAMDE, JAJU 3077, C65-3, STV 213T2 2007plot 300, Rhyne High Sat-1, SG 747, L3, GVS 5069, GVS 3 STV140

Table 7. D2 statistics among different clusters

Cluster	1	2	3	4	5
1	0.00				
2	39.51	0.00			
3	27.27	29.22	0.00		
4	19.53	20.12	20.63	0.00	
5	53.12	87.38	61.55	69.71	0.00

quality. The genotypes in cluster 1 and 5 may be combined to obtain desirable traits related to earliness and better disease tolerance.

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