



Assessment of genetic variation against CLCuD in introduced USDA cotton germplasm

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Abstract. In present study 68 cotton genotypes imported from USA were evaluated for cotton leaf curl virus disease tolerance, monopodia per plant, sympodia per plant, plant height and number of bolls per plant. For evaluation of these traits cluster analysis was employed to obtain suitable parents that can be further used in future breeding programmes for CLCuD tolerance. In addition to these, analysis frequency distribution was also generated to summarise the results on the basis of CLCuD tolerance percentage. For cluster analysis the genotypes were grouped into three clusters (cluster I, II and III) comprised of genotypes with different morphological characters. Cluster II was characterized by genotypes having less attack of CLCuD. Cluster I was represented by genotypes having good plant height and sympodia per plant. Cluster III was characterized by genotypes having high attack of CLCuD, therefore not recommended against CLCuD. Cluster II genotypes are good for CLCuD tolerance and can be utilized in breeding programmes for better CLCuD tolerance. The lowest virus percentage was recorded on the accessions USG16_3098, USG16_3123 and USG16_3089, while the highest virus percentage was recorded on the accessions USG16_3083, USG16_3080 and USG16_3085 as shown by frequency distribution graph. So, it is concluded that the genotypes with lowest CLCuD percentage might be used in breeding programme for the development of CLCuD tolerant varieties.

Key Words: Cotton, Screening, CLCuD, morphological traits, cluster and correlation analysis.

Introduction. The environmental catastrophes, including biotic and abiotic stresses, are the major threats to agriculture and food security (Saeed et al 2015). Biotic stresses including plant viruses, caused many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world, including Pakistan. Cotton leaf curl disease is among one of the most destructive natural disasters that caused massive losses to cotton crop productivity especially in Pakistan during the last 22 years (Farooq et al 2011). This disease caused huge losses not only to the cotton crop but also to the economy of Pakistan (Farooq et al 2014). In 1967 CLCuV diseases was found on few cotton plants first time in Pakistan near Multan (Hussain & Ali, 1975). Later in 1988 the disease was spread rapidly and infected about 24 ha of cotton crop. CLCuV disease has started to become the major problem for cotton as in 1990, 800 ha and in 1992 about 120,000 ha were affected by this disease which caused and 30% reduction in production (Mansoor et al 1993). This figure of infection was increased every year, 400,000 ha were affected during 1993-94 and further reduction in cotton resulted production. Due to appearance of Cotton Leaf Curl Virus disease production was dropped to 7.9 million bales in 1994 (Mahmood et al 2003). Cotton leaf curl virus (CLCuV) belongs to genus *Begomovirus* and family Geminiviridae, and it is a single stranded DNA virus and is transmitted by a sucking insect, whitefly (Iqbal et al 2014). Cotton leaf curl

disease (CLCuD) complex is a debilitating disease of cotton that results in leaf curling, darkened veins, vein swelling and enations that frequently develop into cup-shaped and leaf like structures on the undersides of leaves which causes reduction in cotton yield and fiber quality (Sattar et al 2013; Saeed et al 2014). Among morphological characters plant height, number of bolls, monopodia branches and sympodia branches were severely affected by CLCuD (Farooq et al 2013). Later on, tolerant varieties like CIM-240 and MNH-147 were released by Central Cotton Research Institute Multan (CCRI) and Cotton Research Station Multan (CRS) respectively (Iqbal et al 2014). Losses due to CLCuD were minimized to restrained level and cotton yield remained in between 8-11.5 million bales. Consistent and distressing efforts of cotton breeders eventually lead to a bumper crop yield of 14.5 million bales in 2004-05 before the onset of a new and mutant species of CLCuD known as Burewala virus (Amin et al 2006).

Efforts of the breeders though helped to solve this problem by developing resistance/tolerant varieties using different conventional breeding approaches, but these varieties become susceptible after two to three years due to mutations occurring in the viral strains causing CLCuD. Therefore, sufficient genetic variability, proper exploitation of the existing varieties through hybridization, polyploidy creation and introduction of new exotic germplasm are very important (Esmail et al 2008). The magnitude of variability can be estimated using cluster analysis (Bajracharya et al 2006; Saeed et al 2013). The present research, to estimate the genetic diversity among the exotic lines of *Gossypium hirsutum* L. imported from the USA using cluster analysis was thus conducted to identify suitable genotypes having resistance/tolerance against CLCuD with good morphological traits which may be combined in breeding programmes aimed for the development of virus tolerant genotypes.

Material and Method

The experiment was conducted at the Cotton Research Institute, Faisalabad, Punjab, Pakistan during 2016-17. Sixty- eight genotypes of *Gossypium hirsutum* imported from United States of America were tested against CLCuD incidence. Virus infestation is more severe on late sowing cotton crop in Pakistan due to frequent attack of white fly (*Bemisia tabaci* G.). So, to attain the effective screening against CLCuD, these genotypes were sown late on June 4th, 2016. Row to row and plant to plant distance were kept at 75 cm and 30 cm respectively. All recommended agronomic and cultural practices (hoeing, irrigation, weed eradication and fertilizer application) were carried out. For measuring the morphological data 10 plants and for CLCuD incidence all individual plants were selected. Data were recorded for monopodia per plant, sympodia per plant, plant height and CLCuD infestation. Data on monopodia and sympodia were taken by counting the number of vegetative and fruiting branches. Data of plant height in centimetres were recorded from the base of the plant to the tip of the plant at maturity. CLCuD data were recorded at 30, 60, 90 and 120 days after sowing. CLCuD (%) and the reaction of the accessions was determined following the disease scale described by Akhtar et al (2010) and Farooq et al (2011). The disease scale is given in Table 1. The % age of CLCuD incidence was calculated by using the following formula:

$$\text{CLCuV disease incidence (\%)} = \frac{\sum(\text{all disease ratings})}{\text{total no. of plants}} \times 25$$

Table 1

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

<i>Symptoms</i>	<i>Disease rating</i>	<i>Disease index (%)</i>	<i>Disease reaction</i>
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 enation or both	3	30–50	Susceptible
Stunting alone with, vein thickening leaf curl/enation	4	>50	Highly susceptible

Statistical analysis. The average data were subjected to cluster analysis using statistical software packages of SPSS version 19 and Statistix version 9.0 (Sneath and Sokal 1973) respectively. Cluster analysis was performed using K-means clustering while a tree diagram based on elucidation distances was developed by Ward's method.

Results and Discussion

Cluster Analysis. By using cluster analysis 64 exotic genotypes were grouped into 3 clusters on the basis of various traits (Table 2) and graph of cluster analysis is given in Figure 1. Cluster I, II and III comprised of 24, 25 and 19 genotypes respectively. The genotypes in the 1st cluster represent reasonable values for plant height, monopodial branches and sympodial branches per plant.

Table 2

Cluster analysis of CLCuD and morphological traits in exotic genotypes of cotton

<i>Variable</i>	<i>Cluster-I</i>	<i>Cluster-II</i>	<i>Cluster-III</i>
Plant height (cm)	103	40	70
Monopodia per plant	2.4	1	0
Sympodia per plant	16	15	15
Bolls per plant	0	0	4
CLCuD (%)	30.7	28.2	67.1

The members of 2nd cluster exhibited lower values for CLCuD while plant height, monopodia and sympodia per plant showed also lower values. The cluster III was characterized by genotypes having maximum CLCuD and bolls per plant but lower values for plant height, monopodia and sympodia per plant. The first two principal components contributed almost 34.1 % in total variance. The cluster membership of studied genotypes is given in Table 3.

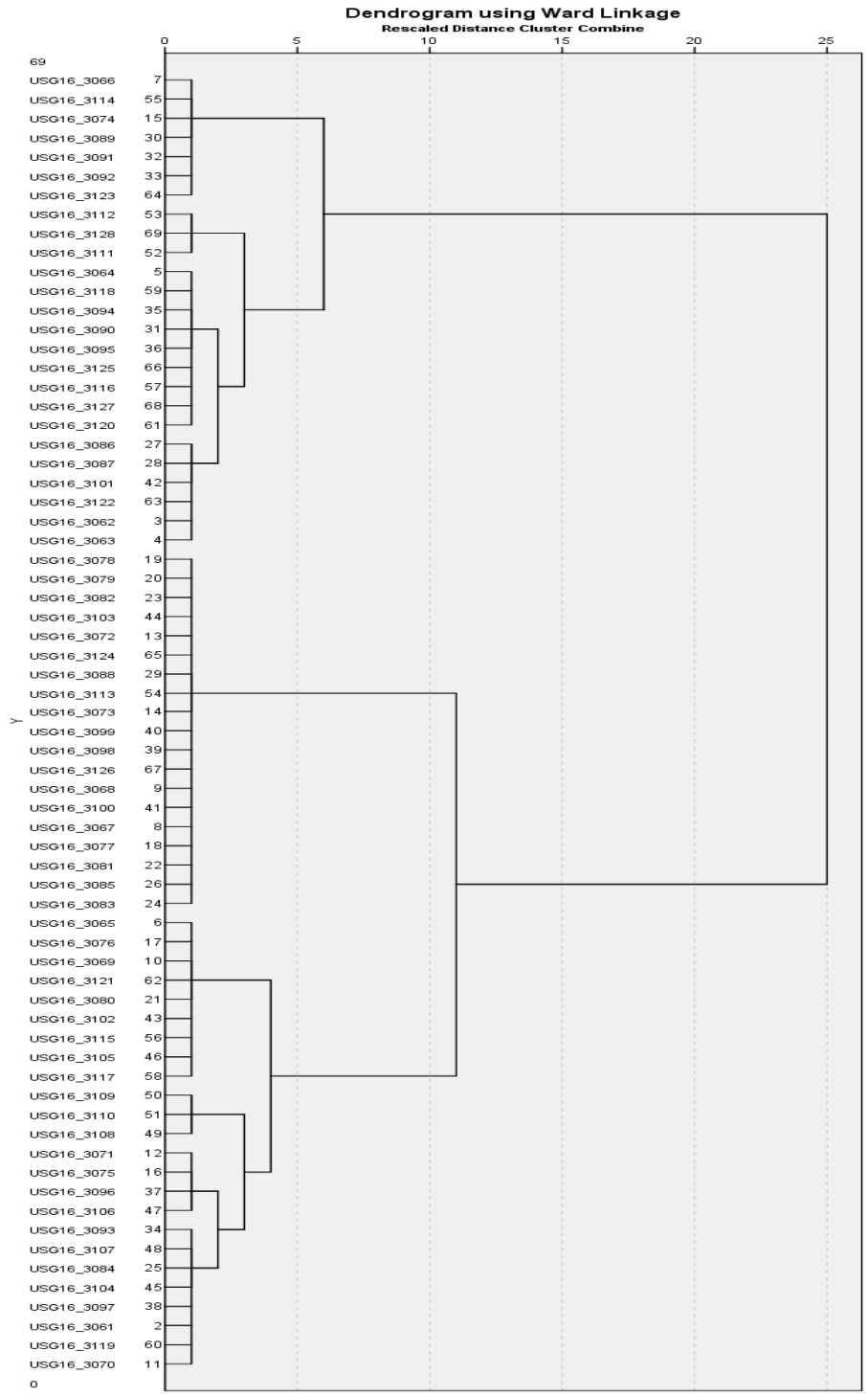


Figure 1. Cluster Analysis of 68 USDA Accessions based on plant height, number of bolls, CLCuD %, monopodia and sympodia per plant.

Table 3

Cluster membership of various genotypes		
Cluster	Accessions No	Name of Accession
Cluster-I	24	USG16_3061, USG16_3065, USG16_3069, USG16_3070, USG16_3071, USG16_3075, USG16_3076, USG16_3080, USG16_3084, USG16_3093, USG16_3096, USG16_3097, USG16_3102, USG16_3104, USG16_3105, USG16_3106, USG16_3107, USG16_3108, USG16_3109, USG16_3110, USG16_3115, USG16_3117, USG16_3119 and USG16_3121
Cluster-II	25	USG16_3062, USG16_3063, USG16_3064, USG16_3066, USG16_3074, USG16_3086, USG16_3087, USG16_3089, USG16_3090, USG16_3091, USG16_3092, USG16_3094, USG16_3095, USG16_3101, USG16_3111, USG16_3112, USG16_3114, USG16_3116, USG16_3118, USG16_3120, USG16_3122, USG16_3123, USG16_3125, USG16_3127 and USG16_3128
Cluster-III	19	USG16_3067, USG16_3068, USG16_3072, USG16_3073, USG16_3077, USG16_3078, USG16_3079, USG16_3081, USG16_3082, USG16_3083, USG16_3085, USG16_3088, USG16_3098, USG16_3099, USG16_3100, USG16_3103, USG16_3113, USG16_3124 and USG16_3126

Frequency Distribution Graph of CLCuD Percentage. The frequency distribution graph of 68 USDA accessions is given in Fig 2. The CLCuD percentage data showed a range of 25.54% to 67.08%. The lowest virus percentage was recorded on the accessions USG16_3098, USG16_3123 and USG16_3089 with the values of 25.54%, 26.95% and 28.21%, respectively, while the highest virus percentage was recorded on the accessions USG16_3083, USG16_3080 and USG16_3085 with the values of 51.19%, 51.25% and 67.08%, respectively.

The variability between clusters is of great genetic importance in finding genotypes aimed at cotton selection for adaptation to virus infested areas. Nazir et al (2013) also found extensive variations in clusters while studying cluster analysis in different cross combinations. Malik et al (2011) and Ashokkumar & Ravikesavan (2011) reported that abundant amount of diversity in coloured cotton genotypes allow opportunities to characterize colour cotton genotypes. Cluster analysis also assisted to elucidate adequate amount of diversity in this set of genotypes at different growth phases. The genotypes in cluster I, II and III comprised of genotypes with better earliness and fiber quality traits. Cluster II was characterized by genotypes having less attack of CLCuD. Cluster I was represented by genotypes having good plant height and sympodia per plant.

Cluster III was characterized by genotypes having high attack of CLCuD, therefore not recommended against CLCuD. Cluster II genotypes are good to utilize for better CLCuD tolerance. The grouping of genotypes in clusters on the basis of various traits in cotton have been reported by Nazir et al (2013).

The existence of inclusive diversity between clusters is of great genetic values that allow selecting the genotypes with wider genetic base for better tolerance against CLCuD and earliness traits. Ayana and Bekele 1999 reported similar kind of results for grouping of germplasm into various clusters. The lowest virus percentage was recorded on the accessions USG16_3098, USG16_3123 and USG16_3089 while the highest virus percentage was recorded on the accessions USG16_3083, USG16_3080 and USG16_3085.

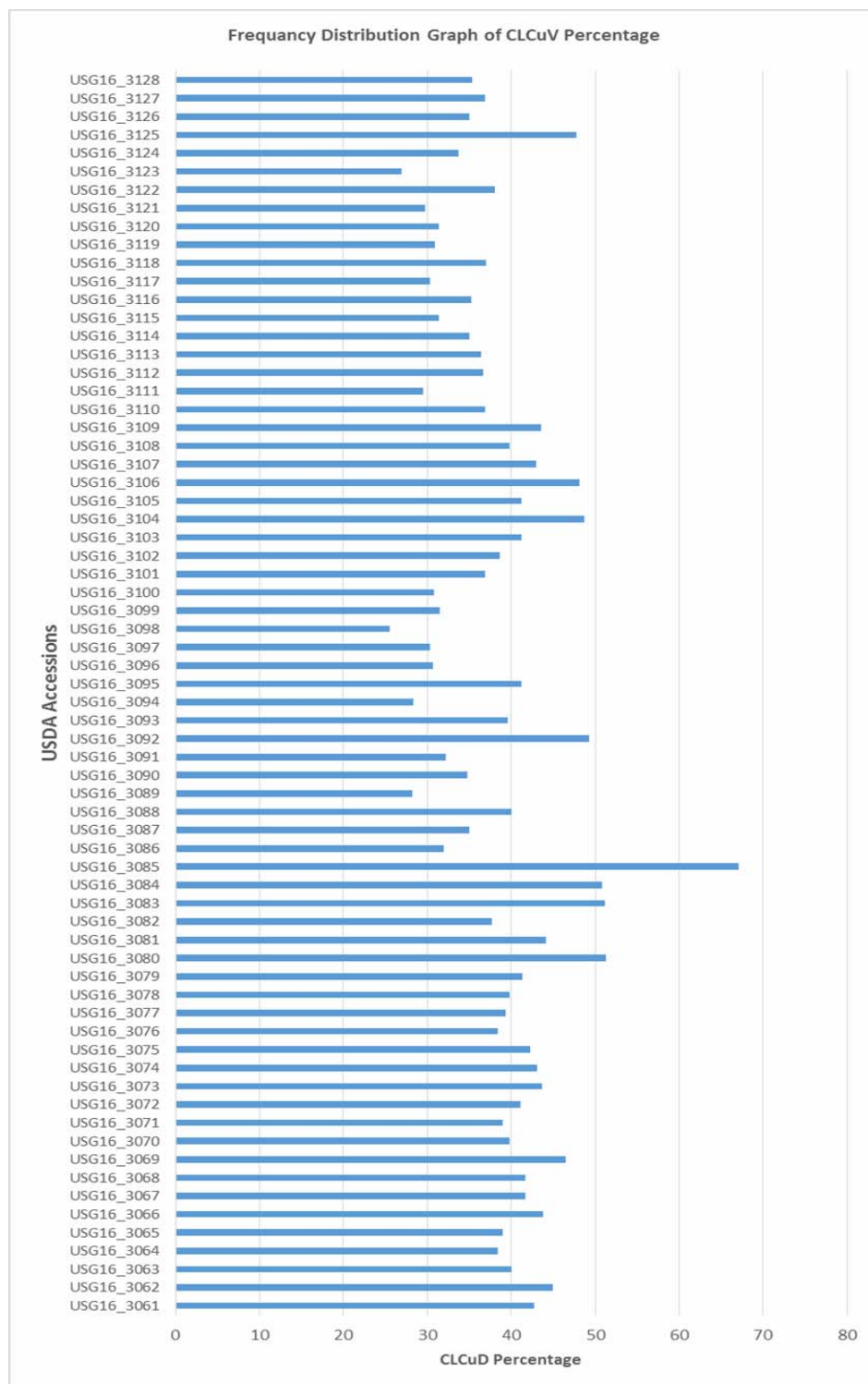


Figure 2. Frequency Distribution of 68 USDA Accessions based on CLCuD Percentage.

Conclusions. The accessions USG16_3098, USG16_3123 and USG16_3089 might be used in breeding programme for the development of CLCuD tolerant varieties. The remaining accessions were not suitable for our climatic conditions.

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