Original Research Article

ASSESSMENT OF GENETIC DIVERSITY FOR COTTON LEAF CURL DISEASE (CLCuD) AND QUALITATIVE TRAITS AMONG ELITE COTTON CULTIVARS

ABSTRACT

Cotton is a major fibrous cash crop and is cultivated in more than 80 countries because global textile industry depends on it. In Pakistan, the major factor reducing the cotton production is cotton leaf curl disease which is caused by a begomovirus, cotton leaf curl virus vectored through a whitefly, *Bemisia tabaci*. This disease shows a wide variety of symptoms including vein thickening, stunted plant growth, and cup-shaped outgrowth known as Enation. This study was conducted to find out the genetic diversity among 50 cotton varieties by using 10 SSR primers. DNA extraction was done by using CTAB method which was followed by DNA quantification through agarose gel to confirm its quantity. PCR and PAGE were performed to check the quality of DNA. Statistical analysis was performed by using Power marker v. 3.25. Primer NAU 2083 showed the maximum genetic diversity and PIC value of 0.8736 and 0.8621, respectively. The unweighted pair-group method analysis (UPGMA) generated the phylogenetic tree to divide the varieties into two clusters which represented the greater genetic diversity in three cotton varieties. These varieties can be utilized in future breeding programmes for cotton improvement.

Keywords: Cotton, CLCuD, Elite, Cultivars, Diversity

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INTRODUCTION

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Cotton (*Gossypium hirsutum* L) is, a major fibrous cash crop and performs a crucial functionutilized in vegetable oil production and forage industries [1]. It is also named as "White Gold" as due to its foreign exchange earnings, consumptions as lint and its other end-products and foreign exchange earnings [2]. ItThe crop is grown widely across the globe because of its economic statusimportance. In many cotton producing countries, cotton diligence is of great worth as it contributes to their GDPs [3]. The upland cotton is accountable for 90% of total world's cotton production [4]. It has yearly production of over 20 million tons and is produced in >more than 80 countries because worldwide textile diligence depends on both natural and artificial sources of fiber production [2]. In agronomic countries like Pakistan, cotton is an significant important economic crop and it subsidizthat contributes about 0.8% in to the Gross Domestic Product (GDP) and 4.5% in the agriculture

division ~~in Pakistan~~of the country [4]. Pakistan is the 5th ~~prevalent~~ major producer and the 3rd prevalent exporter of cotton throughout the entire globe [5]. ~~–~~Economy of Pakistan chiefly depends on cotton fabrication because it is not only the basis of fiber but also offers vegetable oil and low-quality oil for soup diligence. Cotton crop is regarded as the king of fiber and cotton plant delivers 70-80% of natural fiber [2]. Cotton fibers are used for the production of nano-fibrillate cellulose materials via nano-technological appliances. Woven fabrics, foodstuffs, cosmetic and soap are also manufactured from cotton fiber [3]. Edible oil and seed cake are also acquired from cotton seeds [4].

Despite these enormous importance of the crop, ~~Due to~~ several biotic and abiotic ~~causes~~stresses have resulted in low yield of~~,~~ cotton ~~production is reduced~~ [6]. Cotton crop, the cultivated shrub, is susceptible to several pathogens and pests, out of which, the most destructive ~~aspect~~ pathogen is the cotton leaf curl disease (CLCuD) [7]. Cotton leaf curl disease~~CLCuD~~ is caused by multiple begomoviruses which are vectored through the whitefly~~,~~ known as *Bemisia tabaci* (Zubair et al. 2021). Across Pakistan and northwestern India, cotton plants are destroyed by CLCuD causing drastic reduction in production [8]. The objective of study ~~is~~ was to ~~analyze~~ assess the genetic diversity for Cotton Leaf Curl Disease among elite cotton cultivars.

The authors highlighted the statements for their research but could not justify their research.

The authors could not discuss anything on genetic diversity study and its relevance.

Has any work been done previously on the morphological characterization of CLCuD in Pakistan and eslsewhere?

The authors also failed to give background information regarding genetic markers utilized by previous researchers for the study of the disease.

In summary, the authors failed to propose solution to their problem statement, give a brief literature survey and the scope and justification of the work done.

MATERIALS AND METHODS

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Leaf samples of fifty cotton (*G. hirsutum*) varieties were gathered from Cotton Research Station and Central Cotton Research Institute Multan in the months of September and October (Table 1). Susceptible and resistant plants were selected according to the infection scale [9]. For assessment of genetic diversity for CLCuD and phenotypic data –for selected traits was also measured. Fresh and young leaves were selected for genomic DNA extraction via Cetyltrimethyl ammonium bromide (CTAB) method [1]. Extracted DNA was assessed to confirm its quantity through agarose gel electrophoresis**.** –For the evaluation of genetic diversity, SSR primers were used to amplify 50 samples of cotton by using PCR. -PAGE was executed to assess the PCR products.

Table 1: Names of 50 Cotton varieties

Sr. No.	Variety Name	Sr. No.	Variety Name
1	AA 802	**26**	IUB 222
2	Barberton	**27**	LaOkr 541
3	BH 160	**28**	LB 391
4	Bt. CIM 602	**29**	MNH 329
5	CA 325 IRABLT	**30**	MNH 886
6	CEMB 33	**31**	NIAB 112
7	Chilala 76/2	**32**	NIAB 2009
8	CIM 1100	**33**	NIAB 2010
9	CIM 443	**34**	RH 112
10	CIM 446	**35**	S 14
11	CIM 448	**36**	S 32
12	CIM 473	**37**	Samaru 72
13	CIM 482	**38**	SI Okra1 23

14	CIM 499	**39**	Sitar 008
15	Cris 613	**40**	SLH 06
16	DP Acala 90	**41**	SLH 119
17	DPL NEW COTTON 33	**42**	SLH 12
18	FBS 30	**43**	SLH 13
19	FBS 37	**44**	SLH 317
20	FH 113	**45**	SLS 90/2
21	FH 87	**46**	SLS B7/175
22	FVH 53	**47**	TARZAN 1
23	GM 90	**48**	TARZAN 2
24	Gomal 105	**49**	VH 305
25	IR 3701	**50**	VH 363

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Experimental site

Plant materials: The authors should indicate in tabular form the accession number, local name, source (if obtained from different sources), staus (whether breeding line or local material), main attributes

Field trial layout, design, management and evaluation

Genomic DNA extraction and quantification

Molecular data analysis:

How were SSR fragment sizes generated?

Were the generated SSRs converted to binary data using the software "ALS Binary" for statistical analysis (Prasanth and Chandra, 2006)?

WastThe presence (1) or absence (0) of individual allele was scored for each cotton genotype across the 10 SSR markers?

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RESULTS

Analysis of Allele number

For the estimation of genetic diversity among 50 cotton accessions**,** 10 SSR primers were used. The total 57 loci have an average of 5.7 loci per primer. Number of alleles on every primer ranges from 1-8. The total number of alleles amplified by these SSR primers was 57. Primers NAU 980 and BNL 827 exhibited maximum number of polymorphic bands while the minimum numbers of polymorphic bands were exhibited by the primers NAU 2273 and NAU 2437. Primers and their allele number are denoted in the Table 2.

~~Calculating the~~ Allele frequency, Genetic diversity and Polymorphism

Genetic diversity was evaluated by calculating the major allele frequency, polymorphism and allele number using power marker v 3.25. The mean value of genetic diversity was 0.4566 and in these SSR markers, the maximum genetic diversity was demonstrated by the primers NAU 2083 (0.8736) and NAU 2273 (0.6616) whereas the minimum genetic diversity was exhibited by the primers NAU 3414 (0.0000) and NAU 4042 (0.1512) ~~represented by the~~ (Table 3).

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Polymorphism of 10 SSR primers was finding out by calculating the PIC values. Mean PIC value of these primers was 0.4209. Maximum polymorphism was exhibited by the primers NAU 2083 and NAU 2273 having PIC values of 0.8621 and 0.5874, respectively, whereas minimum polymorphism was exhibited by ~~the~~ primers NAU 3414 and NAU 4042 having PIC values of 0.0000 and 0.1471, respectively.

Table 2 Primer, chromosome and allele number

Primers	Chromosome	Number of alleles
NAU 2083	17	7
NAU 2954	2	5
NAU 4042	4	7
NAU 3414	1	5
NAU 5046	5	6
NAU 2838	6	6

NAU 980	5	8
BNL 827	7	8
NAU 2273	3	2
NAU 2437	3	3
Total		57

Table 3 Major Allele Frequency, Gene diversity and Polymorphism

SSR Marker name	Major Allele Frquency	Allele No	Gene Diversity	Polymorphic Information Content
NAU 2083	0.2400	17.0000	0.8736	0.8621
NAU 2954	0.8400	2.0000	0.2688	0.2327
NAU 4042	0.9200	4.0000	0.1512	0.1471
NAU 3414	1.0000	1.0000	0.0000	0.0000
NAU 5046	0.5000	5.0000	0.6344	0.5738
NAU 2838	0.6000	6.0000	0.5856	0.5452
NAU 980	0.7600	5.0000	0.3944	0.3619
BNL 827	0.6400	7.0000	0.5552	0.5258
NAU 2273	0.3800	3.0000	0.6616	0.5874
NAU 2437	0.7000	3.0000	0.4408	0.3728
Mean	0.6580	5.3000	0.4566	0.4209

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Phylogenic tree

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The unweighted pair group method (UPGM) was used for the assessment of genetic diversity among 50 cotton genotypes by using SSR primers. Nei and Li's 1973 bootstrap neighbor joining method generated the phylogenic tree. This phylogenic tree classified the 50 cotton genotypes into two main clusters A and B. Cluster A comprises of 8 cotton genotypes and classified into two sub-clusters L and M. Sub-cluster L contains two sub-sub clusters L1 and L2. Sub-sub cluster L1 denoted three cotton genotypes including LaOkr 541, IR 3701, and NIAB 2010. Sub-sub cluster L2 comprises two cotton genotypes SLH 119 and SLH 13. Sub-cluster M further differentiates into two sub-sub clusters M1 and M2. M1 denoted the one genotype CEMB 33 whereas M2 designated the two cotton genotypes TARZAN 1 and FH 113.

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Cluster B comprises of 42 cotton genotypes and distinguished further into two sub-clusters D and E. Sub-cluster D distributed into two sub-sub clusters D1 and D2. Sub-sub cluster D1 indicated the two cotton genotypes NIAB 112 and AA 802 while D2 exhibited the only one genotype NIAB 2009. Sub-cluster E distinguished into two sub-sub clusters E1 and E2. E1 further classified into E1-a comprising three cotton genotypes FBS 30 and VH 305 and SLH 12 in a distinct group. E1-b contains three genotypes Samaru 72 in one group and the other two genotypes Sitar 008 and MNH 886 in a distinct group. E2-a comprises only one genotype LB 391 however E2-b further distributed into two groups E2-ba and E2-bb. E2-ba comprising 7 genotypes further distinguished into two groups. One group comprises four genotypes Gomal 105, DPL NEW COTTON 33 and SI Okra1 23 and TARZAN 2 in a distinct group. The second group comprises three genotypes BH 160, FBS 37 and Cris 613. E2-bb classified into two groups E2-bb1 and E2-bb2. E2-bb1 further classified into two groups. One group comprising 9 genotypes include S 32, CIM 446, DP Acala 90, CIM 482, Bt. CIM 602, FH 87 and an isolated group of SLH 317, RH 112, and MNH 329. The second group comprises two genotypes VH 363 and SLSB 7/175. E2-bb2 further distributed into two sub-groups. One sub-group comprising five genotypes including CIM 448, CIM 443, CIM 1100 and Barberton and the other S 14 in a distinct group. Second group of E2-bb2 again discriminated into two sub-sub groups. One sub-sub group comprises only 1 genotype CA 325 IRABLT and the other sub-sub group further distinguished into two groups one comprising three genotypes FVH 53, CIM 449 and SLS 90/2 and the other sub-sub group also contains three genotypes GM 90, CIM 473 and Chilala 76/2.

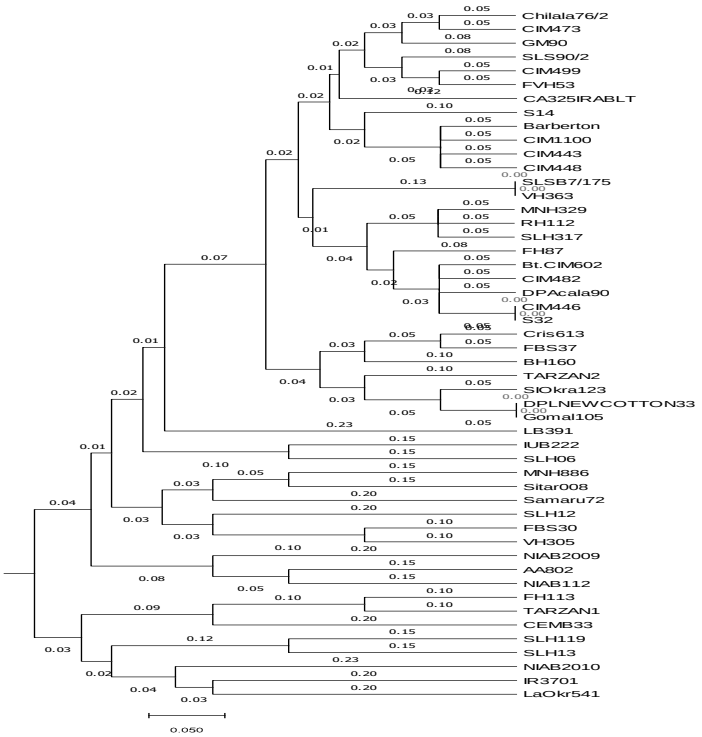

Figure 1: Phylogenic tree of 50 cotton varieties

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DISCUSSION

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Cotton is a substantial fibrous crop which donates in the textile diligence and also the basis of cotton seed. But cotton fabrication is in danger due to various biotic and abiotic reasons. Apart from the abiotic factors, Cotton leaf curl disease caused by *Begomovirus* and vectored through *B. tabaci* declines the cotton yield on the basis of severity of infection. Broad genetic diversity of genotypes can control such diseases [10]. Genetic diversity is defined as the total number of alleles with variable frequencies within a species. Genetic diversity arises due to alterations in the alleles of genetic makeup and is involved in the marker-aided

selection. SSR markers are used for the estimation of genetic diversity in cotton genotypes. These markers are co-dominant in nature and reveal accurate results [11]. Genetic variability for favorable traits is very little among elite cotton accessions. For the development of desirable cotton germplasm, SSR markers are utilized in the marker-aided selection [10].

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In our study, the genetic diversity of 50 cotton genotypes was assessed by using ten SSR primers and total 57 alleles were amplified which ranged from 8 to 2 alleles with an average of 5.7 alleles per primer. Maximum numbers of polymorphic bands were characterized by the primers NAU 980 and BNL 827 whereas minimum numbers of polymorphic bands were demonstrated by the primers NAU 2273 and NAU 2437. In current study, 10 SSR primers were used to observe major allele frequency, genetic diversity and polymorphic information content (PIC) among 50 cotton genotypes. Our results revealed that maximum genetic diversity of 0.8736 and maximum PIC values of –0.8621 were presented by the primer NAU 2083. Estimation of genetic diversity by using SSR markers have been discussed in earlier studies [12].

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Phylogenetic tree was generated by Nei and Li's 1973 bootstrap neighbor joining method to evaluate genetic diversity among 50 cotton varieties. This phylogenetic tree classified these 50 varieties into clusters and various sub-clusters to reveal the similarity index. Our results showed that DPL NEW COTTON 33 and Gomal 105 are closely linked and reside in the same group. Varieties CIM 446 and S 32 are also closely related and present in the same group. [13] reported the same results. Phylogenetic tree was constructed by UPGMA revealed genetic diversity among clusters. SSR primers are beneficial used for the genetic evaluation of cotton such as genetic diversity and marker-aided selection to choose desirable cotton varieties.

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CONCLUSION

This study discovered the genetic diversity by using SSR markers among selected cotton cultivars. ~~From this study, it is find out that great~~The useful genetic diversity ~~is~~

~~recogniz~~detect~~ed~~ in ~~some~~ genotypes Chilala 76/2, Cris 613 and LaOkr 541~~of cotton~~ could be exploited for the genetic improvement of the crop. ~~It is observed that greater genetic diversity is examined in genotypes Chilala 76/2, Cris 613 and LaOkr 541 as they are present on maximum distance in clusters.~~ This assessment of genetic diversity in different cotton genotypes provides the opportunity to the breeders to select parental lines for the production of new cotton genotypes with superior yield and quality.

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