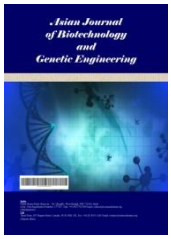

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Assessment of Genetic Diversity for Cotton Leaf Curl Disease (CLCuD) and Qualitative Traits among Elite Cotton Cultivars

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cotton is a major fibrous cash crop cultivated in over 80 countries because the global textile industry depends on it. In Pakistan, the primary factor reducing cotton production is cotton leaf curl disease caused by a begomovirus, a cotton leaf curl virus vectored through the whitefly, *Bemisia tabaci*. This disease shows various symptoms, including vein thickening, stunted plant growth, and cup-shaped outgrowth known as Enation. This study was conducted to analyze the genetic diversity for cotton leaf curl disease among 50 cotton varieties using 10 SSR primers. The results showed that 57 alleles were identified, averaging 5.7 alleles per primer. Maximum Polymorphism was exhibited by the primers NAU 2083 and NAU 2273, having PIC values of 0.8621 and 0.5874, respectively. Phylogenetic tree by neighbor-joining method showed a greater genetic diversity among the cotton genotypes under study suggesting that these cotton varieties can be utilized in future breeding programs for cotton improvement.

Keywords: Cotton; CLCuD; elite; cultivars; diversity.

1. INTRODUCTION

Cotton (*Gossypium hirsutum L*), a major fibrous cash crop, performs a crucial function in oil and forage industries [1]. It is also named "White Gold" for its consumption as lint, end-products, and foreign exchange earnings [2]. It is grown widely across the globe because of its economic

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status. In many cotton-producing countries, cotton diligence is of great worth as it contributes to their GDPs [3]. The upland cotton accounts for 90% of the world's cotton production [4]. Its annual production is over 20 million tons and 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 a significant crop, and it subsidizes 0.8% of the Gross Domestic Product (GDP) and 4.5% in the agriculture division in Pakistan [4]. Pakistan is the fifth most prevalent producer and the third most prevalent exporter of cotton throughout the entire globe [5]. The 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. The cotton crop is regarded as the king of fiber, and the cotton plant delivers 70-80% of natural fiber [2]. Cotton fiber is used to produce nano-fibrillate cellulose materials via nano-technological appliances. Woven fabrics, foodstuffs, cosmetics, and soap are also manufactured from cotton fiber [3]. Edible oil and seed cake are acquired from cotton seeds [4]. Due to several biotic and abiotic causes, cotton production is reduced [6]. The cotton crop, a cultivated shrub, is susceptible to several pathogens and pests, out of which the most destructive is cotton leaf curl disease (CLCuD) [7]. CLCuD is caused by multiple begomoviruses, which are vectored through the whitefly, *Bemisia tabaci* [8]. Across Pakistan and northwestern India, cotton plants are destroyed by CLCuD, causing a drastic reduction in production [8]. Cotton scientists have used SSR markers to evaluate the genetic diversity among various accessions for CLCuD resistance. However, the local germplasm is not that much explored. This study analyzes the genetic diversity for Cotton Leaf Curl Disease among elite cotton cultivars.

2. MATERIALS AND METHODS

Fifty cotton genotypes (Table 1), kindly provided by the Central Cotton Research Institute (CCRI), were grown during the cropping season 2021-22. The plants were grown in Randomized Complete Block Desing, and plant-to-plant distance was maintained at 30 inches. Conventional cultural practices were carried out during the complete crop cycle. Morphological and yield traits were also recorded for all the genotypes. Phenotypic data for selected traits were recorded to measure the genetic diversity for CLCuD. The fifty genotypes were scaled from 0-9 based on disease susceptibility [9].

2.1 DNA Extraction and PCR

For DNA extraction, fresh and young leaves were taken from the terminal branches of the 50 cotton genotypes. DNA extraction was performed using the CTAB method with few modifications [1]. The quality and quantity of the DNA were assessed by resolving 2µl of each sample on 1% agarose gel. The crisped and thicker band shows better quality and quantity. 10 SSR markers were applied to 50 cotton genotypes to explore the genetic diversity. The PCR reaction was carried out in a reaction mixture of 20 µl containing 2 µl DNA template, 4 µl dNTPs, 2 µl of forward and reverse primers, 10 µl PCR buffer, and ddH_2O.

The PCR was carried out for 25 cycles comprising denaturation at 98°C for 10 sec, Annealing at 56-60 °C for 30 sec, and extension at 72°C for 30 sec. The amplified PCR products from each of the SSR primer was resolved on an 8% polyacrylamide gel electrophoresis (PAGE) system. The gel was stained using an ethidium bromide solution, and the gel was pictured using a gel documentation system. The presence or absence of an allele in cotton genotypes was marked as '1' or '0', respectively, across the 10 SSR markers. Genetic diversity was evaluated by calculating the significant allele frequency, Polymorphism, and allele number using power marker v 3.25.

3. RESULTS

3.1 Analysis of Allele Number

For the estimation of genetic diversity among 50 cotton accessions**,** 10 SSR primers were used. A total of 57 loci were discovered, with an average of 5.7 loci per primer. The number of alleles ranged from 1-8 per primer. Maximum Polymorphism was explored by the SSR marker NAU 980 and BNL 827, whereas minimum Polymorphism was explored by the SSR primer NAU 2273 and NAU 2437, respectively. SSR primes with their number of alleles are given in Table 2.

3.2 Allele Frequency, Genetic diversity, and Polymorphism

The mean value of genetic diversity was 0.4566. In these SSR markers, the maximum genetic

diversity was demonstrated by the primers NAU 2083 (0.8736) and NAU 2273 (0.6616). In contrast, the minimum genetic diversity was exhibited by the primers NAU 3414 (0.0000) and NAU 4042 (0.1512) (Table 3).

The mean value for the Polymorphism Information Content (PIC) of 10 SSR 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. In contrast, minimum Polymorphism was exhibited by the primers NAU 3414 and NAU 4042, having PIC values of 0.0000 and 0.1471, respectively.

Table 1. List of 50 Cotton varieties used in this study

Serial. No.	Variety Name	Serial. 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

Table 2. List of primers, chromosome number, and the number of alleles

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

Marker	Major.Allele.Frequency	Number of alleles	Gene Diversity	PIC
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

3.3 Phylogenic Tree

The unweighted pair group method (UPGM) was used to assess genetic diversity among 50 cotton genotypes using SSR primers. Nei and Li's 1973 bootstrap neighbor-joining method was used to generate the phylogenic tree. This phylogenic tree grouped the cotton genotypes into two main clusters, A and B. Cluster A comprises eight cotton genotypes and is classified into two sub-clusters, L and M. Sub-cluster L contains two sub-sub clusters, L1 and L2. Sub-sub cluster L1 contains LaOkr 541, IR 3701, and NIAB 2010, whereas Sub-sub cluster L2 contain 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.

Cluster B comprises 42 cotton genotypes and is distinguished further into two sub-clusters D and E. Sub-cluster D is 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 only one genotype, NIAB 2009. Sub-cluster E is distinguished into two sub-sub clusters, E1 and E2. E1 is further classified into E1-a, comprising three cotton genotypes, FBS 30, VH 305, and SLH 12, in a distinct group. E1-b contains three genotypes Samaru 72, in one group,, and the other two, Sitar 008 and MNH 886, in a distinct group. E2-a comprises only one genotype, LB 391; however, E2-b is further distributed into two groups, E2-ba and E2-bb. E2-ba comprises seven genotypes further distinguished into two groups. In a distinct group, one group comprises four genotypes Gomal 105, DPL NEW COTTON 33, SI Okra1 23, and TARZAN 2. The second group comprises three genotypes BH 160, FBS 37, and Cris 613. E2-bb is classified into two groups E2-bb1 and E2-bb2. E2-bb1 is further classified into two groups. One group comprising nine genotypes includes S 32, CIM 446, DP Acala 90, CIM 482, and 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 is further distributed into two sub-groups. One sub-group comprises five genotypes, including CIM 448, CIM 443, CIM 1100; and Barberton, and the other S 14 in a distinct group. The second group of E2-bb2 again discriminated into two sub-sub groups. One sub-sub group comprises only one genotype CA 325 IRABLT. The other sub-sub group is 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.

4. DISCUSSION

Cotton is a substantial fibrous crop that donates in the textile diligence and the basis of cotton seed. However, 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 based on the 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 genetic makeup and can be exploited using marker-aided selection. Co-dominant SSR markers are used to estimate genetic diversity in cotton genotypes as they reveal accurate results [11]. Genetic variability for favorable traits is very low among elite cotton accessions. For the development of desirable cotton germplasm, SSR markers are utilized in the marker-aided selection [10].

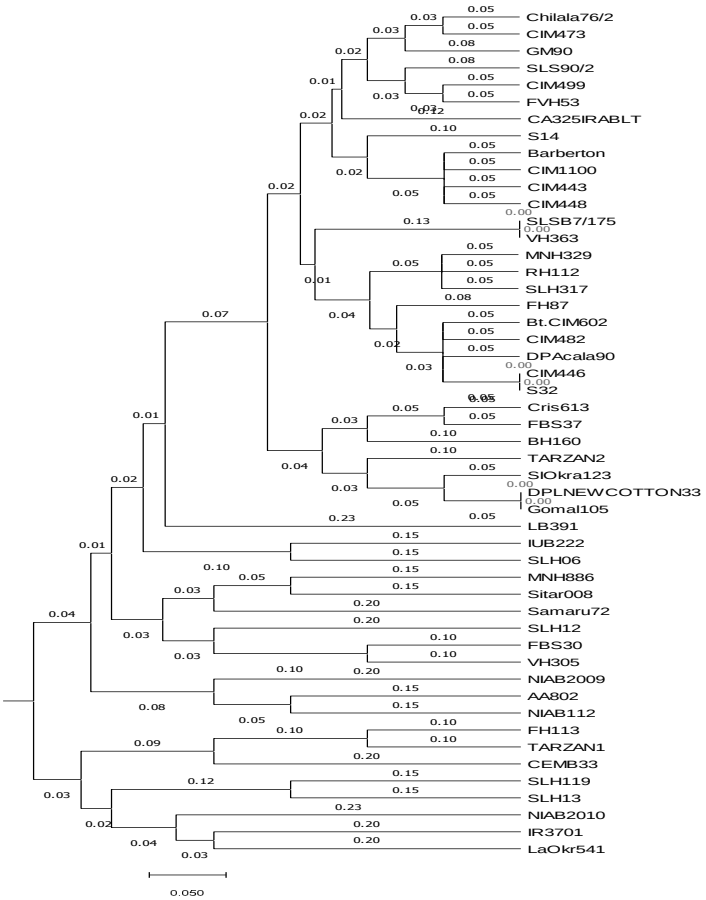

Fig. 1. Phylogenic tree of 50 cotton varieties. The unweighted pair group method (UPGM) was used to construct the bootstrapped phylogenetic tree

Our study assessed the genetic diversity of 50 cotton genotypes using ten SSR primers. A total of 57 alleles were amplified, 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 amplified by NAU 2273 and NAU 2437. In the current study, 10 SSR primers were used to observe significant allele frequency, genetic diversity, and the 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. Estimating genetic diversity using SSR markers has been discussed in earlier studies [12].

Nei and Li's 1973 bootstrap neighbor-joining method generated a phylogenetic tree to evaluate genetic diversity among 50 cotton varieties. This phylogenetic tree classified these 50 varieties into clusters and 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. UPGMA constructed a phylogenetic tree that revealed genetic diversity among clusters. SSR primers are beneficially used for the genetic evaluation of cotton, such as genetic diversity and marker-aided selection, to choose desirable cotton varieties.

5. CONCLUSION

It is necessary to explore the genetic diversity of the cotton germplasm as it is the base of any cotton breeding program. This study analyzed the genetic diversity using SSR markers among selected cotton cultivars. From this study, it is found that great genetic diversity is recognized in some genotypes of cotton. It is observed that greater genetic diversity is examined in genotypes Chilala 76/2, Cris 613, and LaOkr 541

as they are present at a maximum distance in clusters. This assessment of genetic diversity in different cotton genotypes allows the breeders to select parental lines for producing new cotton genotypes with superior yield and quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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