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Evaluation of Genetic Potential of Cotton Lines against Whitefly Tolerance

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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 (*Gossypium hirsutism L.)* is a primary source of natural fiber, fuel, wood, and oil worldwide and an essential raw material source for the textile industry. Whitefly (*Bemisia tabaci*) is one of the major pests distributed worldwide and has broad genetic diversity. In this research, the genetic diversity in the cotton germplasm was explored against the whitefly infestation. Broad sense heritability is a common approach used to detect the association and inheritance of the target trait. Maximum (PIC 0.96) and minimum (PIC 0.36) polymorphism was explored by the SSR primer NAU 988 and NAU 5121, respectively, with an average value of 0.73. Pair-wise genetic estimation ranged from 0.500 to 1.00. Neighbor-joining (NJ) tree, based on UPGMA (Unweighted pair group method with arithmetic), grouped the genotypes into six main clusters, i.e., A, B, C, D, E, and F. Maximum accessions fall into a single cluster showing low genetic diversity among them. The upland cotton accessions FH 326, SLH 07, FH18, and Cris 541, showed divergence from the rest of the genotypes and might have resistance against the whitefly attack. Our results also explain the utilization of the SSR markers to explore genetic diversity and its utilization in a cotton breeding program.

Keywords: Cotton; whitefly; SSR; diversity; resistance.

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1. INTRODUCTION

Generally, the name cotton is derived from the Arabic word "Quotn" [1]. The cotton crop (*Gossypium hirsutum*) has multiple uses, belongs to the family Malvaceae, and the name of its genus is *Gossypium* [2]. Cotton has eight diploid genomes arranged as A to G and one alloploid genome named 'AD.' Out of 50 species of cotton, only four species are cultivated. *Gossypium hirsutum* and *Gossypium barbadense are tetraploids* known as American cotton, whereas Gossypium *arboreum* and *Gossypium herbaceum* are diploids belonging to Asian cotton. Phylogenic study shows *G. hirsutum* (AD) was a result of the hybridization of 2 diploid species named *G. arboretum* (A) and *G. raimondii* (D) [3]. Pakistan is ranked fifth as a producer and third as an exporter of cotton [4]. India ranked first in production and export, followed by China and USA [5, 6]. In Pakistan, the cotton industry is facing several problems during trading at the international level, such as competition for synthetic fiber, deprived fiber quality, and stumpy revenue primarily due to the outbreak of numerous lethal microbes.

Cotton is attacked by numerous sucking and chewing insect pests. Sucking insect pests damages the plants by sucking essential nutrients of plants, making them flabby, yellowing, and drying plants with low fiber quality; moreover, the sucking pests, specifically the whitefly, act as a vector for various viruses. The chewing insect pest eats the vegetative part of the crop [7]. Viruses cause about 11,00 reported diseases in plants, and more than 30% of DNA viruses are transferred through pests, especially whitefly. Lethal viruses that cause disease in plants normally start a molecular and cellular reaction in vectors of pathogens. It is seen in most cases; it disturbs the disease-causing range of germs. However, the machinery process underlying the exporter disturbs growth, and transportation are poorly understood [1]. Whitefly (*Bemisia tabaci*) belongs to the genus *hemipteran*. Whiteflies are complex species with 34 distinct species, 392 holotypes, 44 cryptic species, and 24 altered biotypes. Whitefly imbibes phloem juice from the cotton plant and excretes honeydew-like sticky liquid on the surface of cotton leaves and bolls. Whitefly act as a vector for many plant viruses; these Begomoviruses belong to Geminiviridare major treat for cultivating upland cotton cultivars. The whitefly has excellent reproductive potential, minute size, wide diversity, board host range, and compliance. Due to their characteristics, whitefly shows tolerance against insecticides, i.e., pyrethroids, organophosphates, acephate, and neonicotinoid, which are used for whitefly management [8]. The most commonly utilized classical methods to check insect potential and application of insecticides. Traditional and biochemical approaches produce parasitoids, such as integrated pest management (IPM) and biological control. However, pathogenic fungi are used as mycoinsecticide to control whitefly attacks. Yet, because of their rapid reproductive potential, they can quickly stun the cotton crop, provoking breeders to use effective doses of insecticides and pesticides when the amount of flies per leaf is few [9].

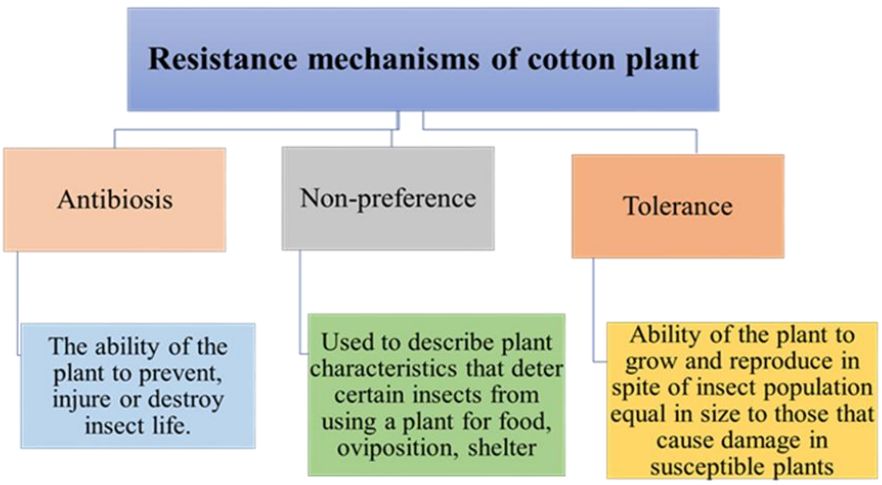

Fig. 1. Resistance mechanisms of a cotton plant

Plants have naturally occurring resistance mechanisms to fight against pathogens name as antibiosis, tolerance, and non-preference, which work about pathogen attacks. Various studies showed induced tolerance in the DNA sequence of cotton crops that are attacked by whitefly. Sometimes, antibiosis and antixenosis work mutually against whitefly attack [10]. Molecular markers such as SSR, RAPD, RFLP, SNP, and next-generation sequencing (NGS) improve cotton varieties against whitefly tolerance. Genome-wide association study (GWAS) has been used in cotton cultivars to evaluate genetic diversity and association mapping, resulting in better quality and quantity of cotton fibers [11]. Based on the above, this research is focused on identifying cotton lines having tolerance against whitefly.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Cotton seed samples were obtained from Central Cotton Research Institute (CCRI) Multan Pakistan and were grown in a Randomized Complete Block design. Fresh Leaves of 50 cotton accessions were collected (Table 1) for DNA isolation in zipper-lock plastic bags and labeled with a black marker. These plastic bags were positioned in an ice box to protect leaf samples from sunlight during traveling, transferred to the lab, and stored at -20℃ until DNA extraction was started. Experimental work was carried out in the Institute of Molecular Biology and Biotechnology Laboratory, Bahauddin Zakariya University, Multan, Pakistan.

2.2 DNA Extraction

To study the cotton genome, total DNA was isolated from leaves of cotton accession using the CTAB method [12] with few modifications.

2.3 DNA Quantification

DNA quantification was carried out by resolving the 2µl DNA sample from each genotype on 1% agarose gel along with a DNA ladder. After confirmation, isolated DNA was stored at -20℃.

2.4 SSR Primers Analysis

Twenty SSR primers were chosen in a way to cover the maximum portion of the cotton genome. SSR primers were selected because they are codominant, multi-allelic, widely spread on the whole genome of the cotton crop, and showed higher PIC value than RAPDs primers. The primer pairs were obtained from different sources such as NAU [13], BNL from Research Genetic Cotton (Huntsville, Al, USA http.\\ www.resgen. com) [14], and JESPER [15].

2.5 Polymerase Chain Reaction

PCR reaction mixture consisted of Template DNA (2µl), master mix (10µl), forward and reversed primers (1.5µl, 1.5µl), MgCl2 (0.5µl), and ddH_2O to make the reaction mixture up to 20µl. Particular SSR primers were carefully

Table 1. List of evaluated genotypes

Sr no	Genotype name	Sr no	Genotype name	Sr no	Genotype name
1	B 021	18	FH 152	35	CRSM 38
2	Barhi M1	19	FH 326	36	GH 99
3	Bt CIM 599	20	FH 941	37	Gomal 93
4	Chandni 95	21	FH 942	38	Hari Dost
5	CIM 496	22	GH 114	39	Malmal
6	CIM 506	23	CIM 632	40	MPS 50
7	CIM 554	24	Cris 541	41	NS 131
8	CIM 573	25	Cris 562	42	NS 181
9	CIM 591	26	Cris 580	43	SADOORI
10	CIM 599	27	Cris 583	44	SH 06
11	CIM 612	28	Cris 587	45	Sindh 01
12	Cyto 124	29	Cris 590	46	SLH 04
13	Cyto 179	30	Cris 599	47	SLH 07
14	FH Lallazzar	31	Cris 601	48	VH 281
15	FH 114	32	Cris 625	49	GH 99
16	FH 118	33	Cris 628	50	VH 282
17	FH 142	34	Cris 635		

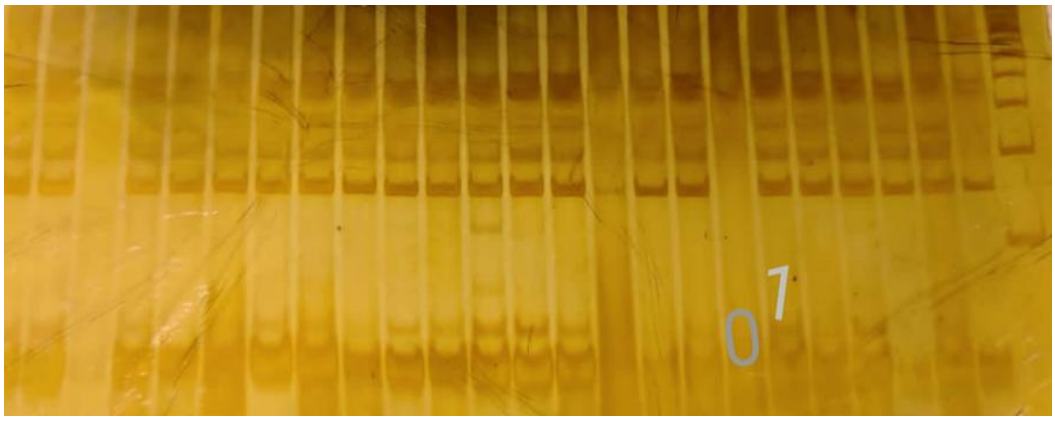

Fig. 2. PAGE gel and its scoring

chosen for specific DNA segments that were liable for whitefly tolerance. PCR amplification profile was programmed for initial denaturation at 94℃ for 7min, followed by 35 cycles of denaturation at 94℃ for 1min, annealing at 55℃ for 1min, and extension at 72℃ for 1min, followed by final extension at 72℃ for 7min. Twenty SSR primers were applied on all 50 cotton accessions [16].

2.6 Polyacrylamide gel Electrophoresis

Poly Acryl Amide gel electrophoresis (PAGE) was used to resolve the PCR products. The liquid mixture of gel consisted of acrylamide solution (11.25ml), ammonium persulfate (400ul),1X buffer (26.25ml), and TEMED (30ul). Ammonium persulfate and TEMED were poured simultaneously as it was helpful for gel polymerization. The liquid solution was poured into glass plates and let polymerize. Electrophoresis was done in 1X buffer at 120v and 70Amp for 1 hour. After the electrophoresis, the gel was silver stained and seen under the illuminator (Fig. 2).

2.7 Data Analysis

The 50 varieties of cotton were categorized based on the absence or presence of the DNA band. The DNA bands were scored manually as '0' or '1' depending on the absence or presence of the target allele, respectively. PowerMarker 3.25 was used to test the genetic diversity, allele number, major allele frequency, genetic distance, and PIC of 50 accessions of the cotton.

3. RESULTS

3.1 Estimation of Allele Numbers

Twenty SSR primer pairs were used to check genetic diversity among 50 cotton genotypes. 20 SSR primers amplified 102 loci with an average of 1.5 loci per primer. The maximum number of alleles, 8, were amplified by SSR primer NAU2083, NAU 883, and NAU 988. The minimum number of alleles, 2, were amplified by SSR primers NAU 5121 and BNL 2443 (Table 2).

Table 2. List of primers, their position, and number of alleles

Name of primers	Number of chromosomes	Total No of alleles	Name of primers	Number of chromosomes	Total No of alleles
NAU 2083	15	8	NAU 2868	11	5
NAU 883	9	8	NAU2838	9	4
BNL 3971	8	4	NAU 980	14	6
BNL 2443	3	2	BNL 827	10	4
BNL 786	7	5	JESPER 274	23	7
NAU 5121	4	2	BNL 4096	7	3
NAU 2954	10	5	JESPER 110	16	5
NAU 1070	21	7	JESPER 153	17	5
BNL 3651	9	4	JESPER 134	16	5
NAU 988	34	8	NAU 3911	7	5
Total volume	12	102			

Table 3. Marker, major allele frequency, allele no, gene diversity, and PIC

Sr no	Marker	Major allele frequency	Allele no	Gene diversity	Pic
1	NAU 2083	0.3600	15.0000	0.8160	0.8001
2	NAU 883	0.5000	9.0000	0.6704	0.6289
3	BNL 3971	0.4400	8.0000	0.7176	0.6793
4	BNL 2443	0.7200	3.0000	0.4392	0.3946
5	BNL 786	0.6600	7.0000	0.5424	0.5229
6	NAU 5121	0.7600	4.0000	0.3976	0.3686
7	NAU 2954	0.5400	10.0000	0.6760	0.6563
8	NAU 1070	0.1800	21.0000	0.9184	0.9131
9	BNL 3651	0.1800	9.0000	0.8592	0.8427
10	NAU 988	0.0600	34.0000	0.9640	0.9628
11	NAU 3911	0.5000	7.0000	0.6600	0.6119
12	J 134	0.3200	16.0000	0.8400	0.8261
13	NAU 2868	0.3600	11.0000	0.8008	0.7793
14	NAU 2838	0.4400	9.0000	0.7568	0.7357
15	NAU 980	0.3600	14.0000	0.8128	0.7955
16	BNL 827	0.3000	10.0000	0.8056	0.7822
17	J 274	0.2000	23.0000	0.9144	0.9090
18	BNL 4096	0.3200	7.0000	0.7856	0.7553
19	J 110	0.3200	16.0000	0.8368	0.8223
20	J 153	0.2400	17.0000	0.8640	0.8514
	Mean	0.3880	12.5000	0.7539	0.7319

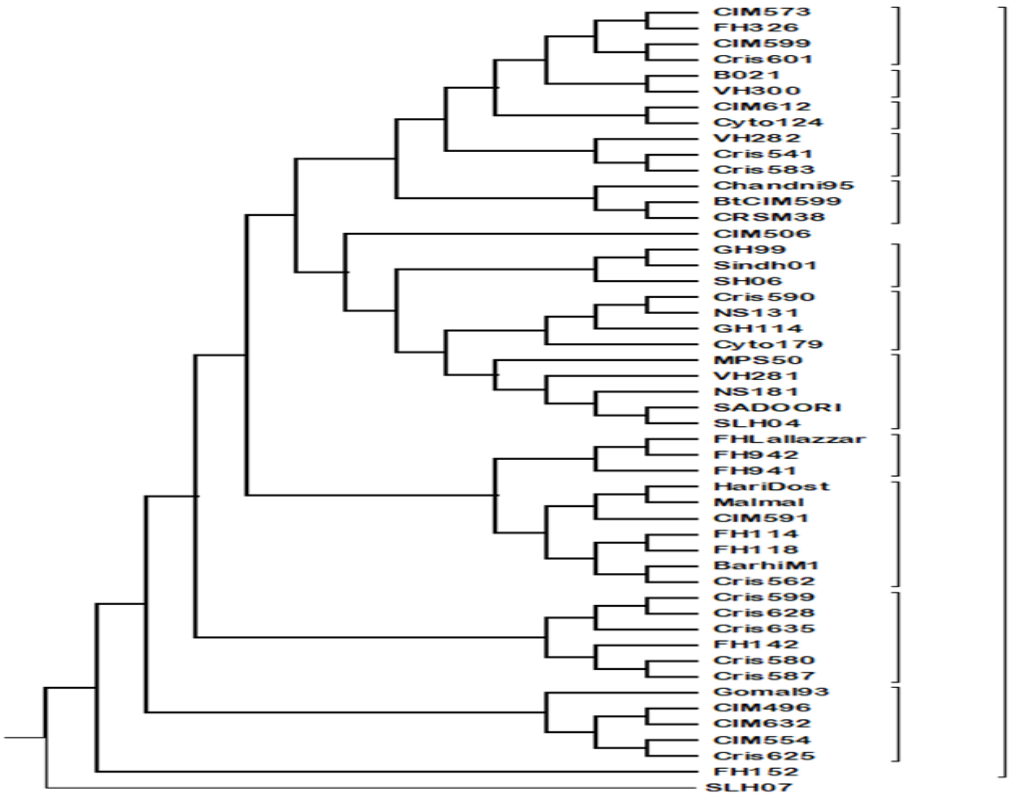

Fig. 3. Triangular form of UPGMA Dendrogram displayed genetic relationship among 50 accession

Table 4. Frequency-based pair-wise similarity among 50 accession

OTU	8021	Sarhi M1	Bt CIM 599	Chandni 35	CIM 496	CIM 506	CIM 554	CIM 573	CIM 591
8021	0.000								
Sarhi M1	0.7000	0.0000							
Bt CIM 599	0.7500	0.9000	0.0000						
Chandni 35	0.8000	0.8500	0.6000	0.0000					
CIM 496	0.9000	0.8500	0.9500	0.9000	0.0000				
CIM 506	0.8500	0.7500	0.9000	0.8500	0.9500	0.0000			
CIM 554	0.8500	0.6500	0.8500	0.9000	0.8000	0.8000	0.0000		
CIM 573	0.6000	0.8500	0.7000	0.7500	0.9000	0.8500	0.9500	0.0000	
CIM 591	0.7000	0.7000	0.7000	0.7000	0.9000	0.8500	0.9000	0.7000	0.0000
CIM 599	0.7500	0.7500	0.6000	0.6000	0.9000	0.8000	0.8000	0.6000	0.7500
CIM 612	0.6500	0.7500	0.7500	0.7500	0.8500	0.7000	0.8500	0.6000	0.7000
CIM 632	0.7500	0.6500	0.8500	0.7500	0.6500	0.8000	0.6500	0.8500	0.8000
Cris 541	0.6500	0.9500	0.7000	0.7000	0.9000	0.9000	1.0000	0.7000	0.7000
Cris 562	0.8500	0.6000	0.8500	0.8000	0.7500	0.9000	0.7500	0.8000	0.6500
Cris 580	0.6500	0.8500	0.8000	0.7000	0.9500	0.8500	0.9500	0.7500	0.6000
Cris 583	0.7000	0.8000	0.7500	0.7500	0.8500	0.7500	0.8500	0.7500	0.8000
Cris 587	0.8000	0.6500	0.9500	0.8500	0.8000	0.9500	0.8500	0.8500	0.7000
Cris 590	0.8500	0.7500	0.8000	0.7500	0.8000	0.7500	0.7000	0.8500	0.8000
Cris 599	0.8000	0.7500	0.8000	0.8000	0.8500	0.8000	0.6000	0.9000	0.9500
Cris 601	0.5500	0.7000	0.7000	0.6500	0.9000	0.8000	0.7000	0.6000	0.7000
Cris 625	0.9000	0.8000	0.8000	0.8500	0.8000	0.8000	0.6500	0.8500	0.8500
Cris 628	0.7500	0.8000	0.8500	0.7000	0.7000	0.8000	0.7000	0.8000	0.9000
Cris 635	0.9000	0.7500	0.9000	0.8000	0.9000	0.7500	0.6500	0.8500	0.8000
CRSM 38	0.7500	0.8000	0.5000	0.6500	1.0000	0.8500	0.8500	0.7000	0.7000
Cyto 124	0.8000	0.7500	0.7000	0.7000	0.9000	0.8000	0.8500	0.7500	0.7500
Cyto 179	0.9000	0.7500	0.8000	0.8000	0.7000	0.6500	0.6500	0.8500	0.8000
FM Lallazzar	0.9500	0.7000	0.6000	0.8000	0.9000	0.7500	0.8500	0.8500	0.7500
FM 114	0.8500	0.6000	0.7000	0.8500	0.9500	0.8500	0.8500	0.7500	0.6000
FM 118	0.8500	0.7000	0.7000	0.9500	0.9000	0.8500	0.9000	0.6500	0.6500
FM 142	0.7000	0.7500	0.8500	0.7500	0.8500	0.8500	0.8000	0.7500	0.8000
FM 152	0.9000	0.7500	0.8500	0.800	0.8500	0.7500	0.8500	0.9000	0.8500
FM 326	0.6000	0.8000	0.5000	0.6500	0.8500	0.9000	0.8000	0.5500	0.7000

OTU	8021	Sarhi M1	Bt CIM 599	Chandni 35	CIM 496	CIM 506	CIM 554	CIM 573	CIM 591
FM 941	0.8000	0.8000	0.7000	0.7000	0.9500	0.8000	0.9000	0.7500	0.8000
FM 942	0.9000	0.8500	0.7000	0.8500	1.0000	0.9000	0.9500	0.7000	0.7500
GM 114	0.9000	0.8000	0.7500	0.8000	0.9500	0.7000	0.8500	0.8500	0.9000
GH99	0.8000	0.7500	0.7000	0.800	0.9500	0.8000	0.7500	0.7500	0.9000
Gomal 93	0.9000	0.7500	0.9000	0.7000	0.9000	0.8000	0.8500	0.9000	0.9000
Mari Dost	0.7000	0.5500	0.8500	0.8000	0.8000	0.7500	0.8000	0.6500	0.6000
Malmal	0.6500	0.6000	0.8000	0.7500	0.8500	0.8500	0.8000	0.7500	0.5000
MPS SO	0.8500	0.7000	0.6500	0.7000	0.9500	0.8000	0.7500	0.8000	0.7000
NS 131	0.8500	0.7000	0.7500	0.7500	0.9000	0.7000	0.7500	0.8500	0.8000
NS 181	0.6500	0.7000	0.7000	0.7500	0.9500	0.6500	0.7500	0.6000	0.7500
SADOORI	0.7500	0.8000	0.6500	0.8500	0.9000	0.7500	0.8000	0.7500	0.7500
SM C6	0.7000	0.7500	0.8000	0.7500	0.9500	0.8000	0.7500	0.7000	0.8000
Sindh O2	0.8500	0.7500	0.8500	0.9000	0.9500	0.7000	0.800	0.7000	0.9000
SLM 04	0.7500	0.7500	0.7500	0.8500	0.8500	0.8000	0.7500	0.7500	0.8000
SLM 07	0.8500	0.9000	0.9000	1.0000	0.8500	0.9500	0.9500	0.7500	0.9000
VH 281	0.8000	0.8000	0.6500	0.8000	0.9000	0.8000	0.8500	0.6500	0.8000
VH 282	0.6500	0.8000	0.6500	0.7500	0.9000	0.7500	0.8500	0.7000	0.6500
VH 300	0.5000	0.6500	0.7000	0.8000	0.9000	0.8000	0.7500	0.5500	0.7000

3.2 Assessment of Allele Number, Genetic Diversity, and Polymorphism Information Content (PIC) Value

PowerMaker 3.25 was used to check genetic diversity among 50 cotton genotypes. The genetic diversity ranged from 0.39 to 0.96, with an average value of 0.75. The maximum level of genetic diversity was explored by NAU 988, while the minimum level of genetic diversity was shown by NAU 5121.

To assess polymorphism level by calculating PIC value with the utilization of 20 SSR primers among 50 cotton accessions. The maximum level of polymorphism shown by NAU 988, its PIC value was 0.96. while a low level of polymorphism was shown by NAU 5121, its PIC value was 0.36. the average value of PIC was 0.73 among 50 cotton genotypes. Most of them used polymorphic SSR primers, but some were monomorphic (Table 3).

3.3 Frequency-based Pair-wise Similarity

A handy software power marker v.325 and method Nei 1973 were used to calculate pair-wise similarity among 50 cotton genotypes. The similarity matrix was arranged from a maximum of 0.50 to a minimum of 1.00. the maximum level of similarity observed by B-021, V 300, CIM 591, and Malmal. In contrast, the minimum similarity was shown by CIM 554, Cris 541, CRMS 38, and CIM 496 (Table 4).

3.4 Phylogenic Tree

The phylogenic tree was constructed using the bootstrap neighbor-joining (NJ) technique based on Nei 1973 method. Fifty cotton genotypes were divided into clusters based on their similarity coefficient. The UPGMA dendrogram made six main clusters named A, B, C. D, E and F. UPGMA is an unweighted pair group method with arithmetic mean. These six main clusters are also divided into a subgroup and sub-subgroups. Clusters A, B, C, D, E, and F contain 27,10, 6, 5, 1, and 1 accession, respectively (Fig. 3).

4. DISCUSSION

Evaluation of genetic diversity amongst cotton cultivars provides essential information that was helpful in the development of diversity and conserve cotton. Molecular markers are used to evaluate genetic diversity and screen the elite genotypes because these markers have a gene segment containing beneficial traits [17]. In this research work, we focused on the effectiveness of SSR markers among 50 accessions of the cotton crop. SSR markers were used because they are multi-allelic, do not require pure template DNA, have a hypervariable nature even among closely related varieties shown allelic variation, and are easily and automatically scored. This research used twenty simple sequence repeat (SSR) primers to evaluate genetic diversity between fifty cotton genotypes. Out of twenty, 80% of SSR primers were polymorphic, while 20% were monomorphic. The aggregate number of alleles amplified by these SSR markers was 102 and the average allele value for each primer was 1.5, which was 73% mutually informative. NAU 2083, NAU 833, and NAU 988, these SSR markers displayed eight bands in the research work. SSR marker named NAU 988 showed the highest level of polymorphism because it displayed 96% polymorphism. In addition, some of them also showed a high level of polymorphism, i.e., NAU 1070, JESPER 274, and JESPER 153, with a PIC value of 91%, 90%, and 85%, respectively.

On the other hand, none of the SSR primers separate overall cotton genotypes. Five SSR primers showed high gene diversity: JESPER 274, NAU 1070, JESPER 153, BNL 3651, and BNL 134, and their gene diversity values were 0.91, 0.91, 0.86, 0.85, and 0.85, respectively. similarity among 50 cotton genotypes was evaluated by PowerMarker v 3.25 [17]. The level of pair-wise similarity was arranged as 0.50 to 1.00. the highest level of pair-wise similarity was observed in cotton genotypes B-021, V 300, CIM591, and Malmal, While the lowest level of pair-wise similarity was observed in cotton genotypes named CIM554, Cris541, CRMS38, and CIM496. UPGMA dendrogram was constructed using the bootstrap neighbor joining (NJ) technique based on an important method named [18]. On the base of the similarity coefficient, fifty cotton genotypes were scattered into different clusters. Main 6 clusters formed among 50 cotton genotypes mentioned as A, B, C, D, E, and F. these six main clusters are also distributed into small groups, sun group, and sub-sub groups. Cotton genotypes CIM 496, CIM632, CIM 554 and Cris 625 showed high genetic relation and were found in the same cluster. On the other hand, CIM 506, MPS 50, and VH 282 share the same group.

5. CONCLUSION

We should not only rely on chemical means for managing whitefly control. Learning and studying genetic diversity helped us to preserve genetic information of whitefly-resistant cotton varieties for better cultivation in the future. Under changing environmental conditions, the evaluation of genetic diversity played a vital role in starting breeding plans, especially for cotton crops. Our study calculated PIC value and pair-wise similarity and constructed UPGMA phylogenic tree to check genetic diversity among 50 cotton genotypes. Most genotypes showed low genetic diversity because they fall in the same group. At the same time, others displayed great genetic diversity because they exist in a diverse group. Our results revealed that cotton genotypes FH 326, SH 07, FH 18, and Cris 541 have great genetic diversity. Therefore, these cotton genotypes are preferred for subsequent breeding and development of new lines of the cotton crop when these lines, different from earlier, showed excellent tolerance against whitefly outbreaks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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