Evaluation of Genetic Potential of Cotton Lines against Whitefly Tolerance

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Article Information
DOI: 10.9734/AJBGMB/2022/v12i230289

ABSTRACT

Cotton (Gossypium hirsutum 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 allopolyploid 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. Phylogenetic 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 imbibles 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 Geminiviridae 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°C 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°C.

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 ddH2O to make the reaction mixture up to 20µl. Particular SSR primers were carefully

| 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     | BI 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      |       |               |

Table 1. List of evaluated genotypes
chosen for specific DNA segments that were liable for whitefly tolerance. PCR amplification profile was programmed for initial denaturation at 94°C for 7min, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min, and extension at 72°C for 1min, followed by final extension at 72°C 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).

| 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| 0.000     | 0.000      | 0.000      | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   |
| Sarhi M1  | 0.700| 0.000     | 0.000      | 0.000      | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   |
| Bt CIM 599| 0.750| 0.900     | 0.000      | 0.000      | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   |
| Chandni 35| 0.800| 0.850     | 0.600      | 0.000      | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   |
| CIM 496   | 0.900| 0.850     | 0.950      | 0.900      | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   |
| CIM 506   | 0.850| 0.750     | 0.900      | 0.850      | 0.950   | 0.000   | 0.000   | 0.000   | 0.000   |
| CIM 554   | 0.850| 0.650     | 0.850      | 0.900      | 0.800   | 0.800   | 0.000   | 0.000   | 0.000   |
| CIM 573   | 0.600| 0.850     | 0.700      | 0.750      | 0.900   | 0.850   | 0.950   | 0.000   | 0.000   |
| CIM 591   | 0.700| 0.700     | 0.700      | 0.700      | 0.900   | 0.850   | 0.900   | 0.700   | 0.000   |
| CIM 599   | 0.750| 0.750     | 0.600      | 0.600      | 0.900   | 0.800   | 0.800   | 0.600   | 0.750   |
| CIM 612   | 0.650| 0.750     | 0.750      | 0.750      | 0.850   | 0.700   | 0.850   | 0.600   | 0.700   |
| CIM 632   | 0.750| 0.650     | 0.850      | 0.750      | 0.650   | 0.800   | 0.650   | 0.850   | 0.800   |
| Cris 541  | 0.650| 0.950     | 0.700      | 0.700      | 0.900   | 0.900   | 0.900   | 1.000   | 0.700   |
| Cris 542  | 0.850| 0.600     | 0.850      | 0.800      | 0.750   | 0.900   | 0.750   | 0.800   | 0.650   |
| Cris 562  | 0.650| 0.850     | 0.800      | 0.700      | 0.950   | 0.850   | 0.950   | 0.750   | 0.600   |
| Cris 580  | 0.650| 0.750     | 0.800      | 0.700      | 0.850   | 0.750   | 0.850   | 0.750   | 0.800   |
| Cris 587  | 0.800| 0.650     | 0.950      | 0.850      | 0.800   | 0.950   | 0.850   | 0.850   | 0.700   |
| Cris 590  | 0.850| 0.750     | 0.800      | 0.750      | 0.800   | 0.800   | 0.800   | 0.600   | 0.900   |
| Cris 599  | 0.800| 0.750     | 0.800      | 0.800      | 0.850   | 0.800   | 0.650   | 0.600   | 0.950   |
| Cris 601  | 0.550| 0.700     | 0.700      | 0.650      | 0.900   | 0.800   | 0.700   | 0.600   | 0.700   |
| Cris 625  | 0.900| 0.800     | 0.800      | 0.850      | 0.800   | 0.800   | 0.650   | 0.850   | 0.850   |
| Cris 628  | 0.750| 0.800     | 0.850      | 0.700      | 0.900   | 0.800   | 0.700   | 0.800   | 0.900   |
| Cris 635  | 0.900| 0.750     | 0.900      | 0.800      | 0.900   | 0.750   | 0.650   | 0.850   | 0.800   |
| CRSM 38   | 0.750| 0.800     | 0.500      | 0.650      | 1.000   | 0.850   | 0.850   | 0.700   | 0.700   |
| Cyto 124  | 0.800| 0.750     | 0.700      | 0.700      | 0.900   | 0.800   | 0.850   | 0.750   | 0.700   |
| Cyto 179  | 0.900| 0.750     | 0.800      | 0.800      | 0.700   | 0.650   | 0.650   | 0.850   | 0.800   |
| FM Lallazzar | 0.950| 0.700    | 0.600      | 0.800      | 0.900   | 0.750   | 0.850   | 0.850   | 0.800   |
| FM 114    | 0.850| 0.600     | 0.700      | 0.850      | 0.950   | 0.850   | 0.850   | 0.750   | 0.600   |
| FM 118    | 0.850| 0.700     | 0.700      | 0.950      | 0.900   | 0.850   | 0.900   | 0.650   | 0.650   |
| FM 142    | 0.700| 0.750     | 0.850      | 0.750      | 0.850   | 0.850   | 0.800   | 0.750   | 0.800   |
| FM 152    | 0.900| 0.750     | 0.850      | 0.800      | 0.850   | 0.750   | 0.850   | 0.900   | 0.850   |
| FM 326    | 0.600| 0.800     | 0.500      | 0.650      | 0.850   | 0.900   | 0.800   | 0.550   | 0.700   |
| 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 | 0.8000 | 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 | 0.8000 | 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 | 0.8000 | 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

PowerMarker 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 pairwise similarity and constructed UPGMA phylogenetic 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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