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Determination of Genetic Divergence Pattern of White Fly Resistant Cotton Cultivars by Using Microsatellite

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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 considered a cash crop in the agriculture sector, and whitefly infestation is one of the cotton crop's significant biotic stresses. This study aimed to identify whitefly Bemisia tabaci resistant cotton varieties that can be incorporated into a cotton breeding program to improve the cotton crop's quality, yield, and growth. In this study, we used 10 Microsatellite (SSR) markers for genetic diversity assessment among 50 genotypes of *G. hirsutum*. 58 loci were found by applying the NAU and BNL series primers. A maximum number of loci, eight, were amplified by SSR markers NAU-883, NAU-2714, and BNL-827, respectively. SSR marker JESPER-101 amplified a minimum number of the loci,i.e., 2. The PIC count ranged from 0.7215 to 0.8828, with a mean value of 0.4034. NAU 2161 displayed a maximum polymorphism value of 0.8828 and BNL 1672 showed a minimum polymorphism value of 0.4034. Cluster analysis grouped the 50 genotypes into four clusters. Cluster A holds 30 varieties. Cluster B includes 11 varieties. Cluster C had six varieties, and cluster D had 3. Genetic diversity is maximum in varieties NS-161, VH-307, and AGC-555, as they are located at the most significant distance in clusters. The SSR genetic profile for every cultivar made it conceivable to separate a few cultivars.

To conclude, this investigation of the genetic divergence of cotton cultivars with SSR markers supports the need to bring new alleles into the genetic pool of the cultivars. It can help in assessing

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the best whitefly-resistant cotton variety. The information generated from diversity analysis studies will help in future breeding plans for improving the hereditary variety of cotton cultivars to fulfill the need for cotton development for various purposes.

Keywords: Bemisia tabaci; SSR markers; Gossypium hirsutum; resistance.

1. INTRODUCTION

Cotton (*Gossypium hirsutum*) is the world's chief natural fibre crop. It is grown in the areas of temperate and tropical climates. Cotton utilization is expanding, compared to a significant expansion in population throughout the planet. Pakistan is a farming nation, and cotton is the second most significant crop contributing a considerable part to the economy. About 1.3 million farmers (out of 5 million) foster cotton on a range of 3 million hectares, covering 15% of the cultivated zone in the country. Cotton and cotton goods account for 1.6% of the GDP and 55 % of the foreign exchange incomes [1]. Cotton is also named as "White Gold" due to its importance in different sectors. Pakistan had a glorious past in terms of cotton production, now facing a decline in production over the years.

The variables for low yield include lack of endorsed seed, pest attacks like whitefly, weed pervasion, incautious utilization of nutrients, and the rate of abiotic stresses (counting dry spell, heat, and salinity). Farmers are reporting from Pakistan that they dread a loss in their cotton crop due to high temperature, rains, and attacks of Bemisia tabaci [2]. The production estimate of the cotton crop has been reduced to 7.4 million bales. The whitefly, *Bemisia tabaci,* is a dangerous pest of numerous vegetables, ornaments, and farming harvests in tropical and subtropical nations of the world [3]. It consumes food from an average of 900 host plants, including species of economic significance related to the 63 families.

Additionally, it also transfers more than 111 plant infections, including cotton leaf curl infection (CLCuD) in American cotton (*Gossypium hirsutum*) [4]. Sucking pests and lepidopteran caterpillar can invade the cotton though out their development cycle. After applying insecticide, the whitefly populace returns shortly as eggs and nymphs swell at foliage's basement and on the lower region of leaves. Sugary material called honeydew secreted by the whitefly draws the sap of cells. The area for photosynthesis is decreased because a black sooty mold happens on the leaves that diminish the crop yield, value, and importance [5]. The attack of *B. tabaci* in cotton has become hard to control with insect sprays because whitefly lives underside the leaves. Moreover, the limited formative period makes them resistant to many insecticides (Organophosphate, Carbamate and Neonicotinoids), and resistant strains have become increasingly bountiful [6].

In order to improve our cotton crop against the whitefly, natural variability and divergence between crops should be broadly identified and distinguished [7]. Studying genetic diversity can help us find the supply of many novel traits presenting tolerance to different biotic and abiotic stresses like whitefly. Diverse lines are required for defect correction of commercial varieties and the establishment of novel varieties. So, identification of diverse lines (if available), formation of diversity (if not available or limited), and its resulting usage are the major areas of any yield improvement programs. Genetic diversity within and between crop plant species allows the breeders to choose superior genotypes to be directly utilized as a new variety or as a parent in a hybridization program.

Different methods are available for genetic diversity analysis. Diverse DNA markers for insect genetics research (i.e., the amplified fragment length polymorphism (AFLP) marker, expressed sequence tags (EST), mitochondrial DNA, microsatellites, and random amplified polymorphic DNA (RAPD) were diagnosed and advanced to decide the populace genetic shape of a species [8]. In this research, we will use microsatellite markers. These markers will be utilized in the cotton improvement for broadening the genetic base and developing varieties against pests and diseases. Microsatellites are particularly famous genetic markers due to their co-dominance, excessive plentiful variant and polymorphism rates, more than one allele, and short allele detection through many methods [9]. Microsatellite markers are also potent in population genetic research for insect species. Through molecular genetic prognosis and the use of populace genetic analyses, powerful manipulation may be carried out quickly at a low cost. Various current researchers have hired

different microsatellite markers to find the populace genetic shape, genetic differentiation, genetic evolution, gene flow, and dispersal sample of *B. tabaci* over extraordinarily massive geographic scales [10].

Microsatellite markers can be used to aid in determining the nature and degree of genetic diversity among inbred lines. It can also help appoint inbred lines effectively to heterotic sets and create heterotic parents' decision to form new hybrids [11]. Pest populace structure tests are beneficial to show the origins and unfold styles of a goal species, to delineate capacity limitations for his or her control, and to offer the statistical capacity to distinguish among genetic groups, in addition to test whether or not they've blended with different populations or not. When all populace genetics records primarily based on microsatellite markers are blended with environmental approaches, the development of an effective framework for dealing with *B. tabaci* is facilitated. Thus, based on the above facts, current research focuses on identifying white fly resistant cultivars for improved cotton yield.

2. MATERIALS AND METHODS

Fifty genotypes (Table-1) of cotton were grown in Cotton Research Institute, Multan, Pakistan, during the cropping year 2020-21. Young leaves were collected from the DNA extraction. Leaf from each genotype was sampled in a separate plastic bag and marked with a permanent marker.

The essential materials required for sampling were small plastic bags and a marker. Leaves were collected in a plastic bag. Marker serves to label the cotton variety from which leaves were taken.

2.1 DNA Extraction, Quantification, and PAGE

DNA was extracted from young cotton leaves by the cetyl trimethyl ammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987) with a few modifications [12]. 2µl of the DNA sample from each cotton genotype was resolved on 1% agarose gel check the DNA samples' quality and quantity [13].

Mullis invented PCR in 1986 to amplify DNA samples [14]. The diversity microsatellite markers (Table-2) were used for the amplification. In 1971, Charmbach and Rodbard came up with the procedure of PAGE (Polyacrylamide gel electrophoresis). PCR products were separated by polyacrylamide gel and electrophoresed. They were then stained with silver nitrate.

PowerMarker software (15) was used for the genetic diversity and the cluster analysis.

Table 1. Varieties of cotton for genetic diversity studies

Variety name	Variety name	Variety name	Variety name	Variety name
1) MNH-552	11) BH-184	21) GH-102	31) VH-307	41) Sahara-150
2) MNH-554	12) RH-647	22) NS-141	32) 1035	42) 1045
3) MNH-147	13) AA-703	23) CIM-707	33) FH-490	43) Sahara Buraq
4)NIAB-Noori	14) Cris-578	24) 1026	34) SS-32	44) IUB-13
5) DNH-105	15) V-14	25) CIM-534	35)Sahara Klean-05	45) BS-15
6) CA-12	16) 1020	26) CIM-608	36) J-05	46) MNH-1086
7) 1016	17) VH-327	27) Bt.CIM-598	37) BS-18	47) BT-A1
8) MNH-129	18) BH-178	28) Sohni	38) N-878	48) AGC- 555
9) RH-662	19) VH-259	29) NS-121	39) Sahara Klean-10	49) NIA-UFAQ
10) RH-668	20) SLH-08	30) SLH-01	40) Sahara-120	50) NS-161

3. RESULTS

In order to analyze the genetic diversity of 50 *G. hirsutum* successions, ten Simple sequence repeat (SSR) marker pairs were employed. By utilizing these ten primers, a sum of 58 loci were established. The highest range of loci was 8. Eight bands were amplified by three markers that were NAU-883, NAU-2714, and BNL-827. The lowest range of loci was 2. Two bands were amplified by one marker, which was JESPER-101. Regarding genetic diversity, the marker that showed maximum genetic diversity was NAU 2161, with a value of 0.8920. 0.4136 was the minimum value of genetic diversity given by the marker BNL 1672. 0.7422 was the mean value of genetic diversity which ranges between 0.8920 and 0.4136 (Table 2).

Table 2. Gene diversity and PIC calculation

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
NAU 2083	0.3200	14.0000	0.8312	0.8146
NAU 883	0.2800	17.0000	0.8256	0.8075
BNL 3971	0.4400	11.0000	0.7512	0.7280
JESPER 101	0.3200	4.0000	0.7360	0.6869
NAU 2161	0.2000	14.0000	0.8920	0.8828
NAU 2714	0.5200	14.0000	0.6984	0.6810
BNL 1672	0.7600	9.0000	0.4136	0.4034
NAU 1070	0.4200	15.0000	0.7856	0.7711
BNL 827	0.2800	17.0000	0.8544	0.8408
BNL 786	0.5600	8.0000	0.6336	0.5994
Mean	0.4100	12.3000	0.7422	0.7215

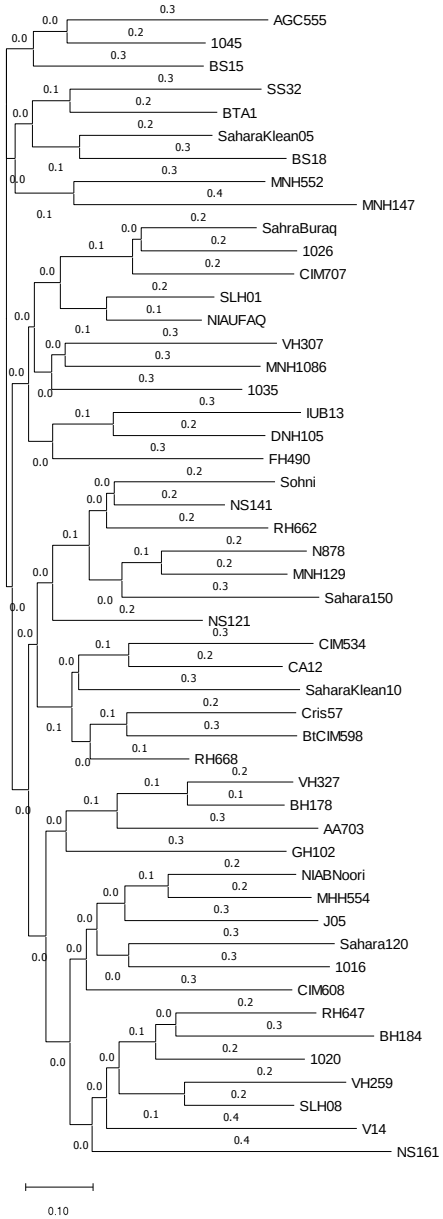

Fig. 1. Dendrogram of 50 cotton varieties

3.1 Similarity Index

In order to find maximum and minimum values of similarity and analyze the relationship among 50 genotypes of cotton varieties, Nei 1973 method from the PowerMarker software was used. The maximal value of genetic distance was 1.00, and the minimum value was 0.5.

3.2 Phylogenetic Tree

PowerMarker software [15] was used to build the phylogenetic tree; the genotypes were grouped into four clusters. These clusters were further divided into sub-clusters and sub-sub-clusters of entire clusters. The main clusters developed were named A, B, C and D. These main clusters undergo more division into sub-clusters and sub-clusters. Cluster A holds 30 varieties. Cluster B includes 11 varieties. Cluster C had six varieties, and cluster D had 3 (Fig. 1).

4. DISCUSSION

The genetic diversity of 50 cotton varieties was explored using 10 Microsatellite markers. These markers formed 58 loci. The NAU-2161 marker expressed the most significant level of polymorphism. In contrast, the BNL-1672 marker exhibited its lowest level. The maximal value of allele number was eight, and it was exhibited by NAU-883, NAU-2714, and BNL-827 markers. 2 was the minimal value of allele number exhibited by JESPER-101. Addressing about genetic diversity, the highest value of 0.8920 was revealed by the NAU-2161 marker. BNL 827, NAU 2083, and NAU 883 also displayed close maximum genetic diversity estimates of 0.8544, 0.8312, and 0.8256, respectively. The most negligible value of genetic diversity was portrayed by BNL-1672, which was 0.4136. The range of genetic diversity is between 0.8920 and 0.41360, with a mean value of 0.7422. To figure out the polymorphism of all 10 SSR markers, polymorphism information content (PIC) analysis was applied. NAU 2161 gave a maximal polymorphism standard of 0.8828. BNL 1672 can be seen to give a minimal standard of polymorphism that was 0.4034. The range of genetic diversity comes between 0.8828 and 0.4034, with a mean value of 0.7215.

Some cotton varieties show maximum similarity, and some show minimum similarity. Genetic diversity is maximum in varieties NS-161, VH-307, and AGC-555 as they are located at the most significant distance in clusters. The maximal value of genetic distance was 1.00, and the minimum value was 0.5 base pairs. The minimum relation was found between BH-178 and AA-703, AGC-555 and 1045, Cris-57, and Bt CIM 598, respectively. The maximum relation was found between wide varieties, including BH-184 and 1045, BS-18 and BH-178, DNH105 and NS-161, respectively. Polymorphic SSRs can be highly informative for molecular genetic diversity studies in various cotton varieties [16].

5. CONCLUSION

Cotton being a cash crop, is very critical for economic security. In this research, we conclude to discover the genetic diversity among the cotton varieties grown in Pakistan. There is a need to utilize this genetic diversity in cotton germplasm to breeding new cotton varieties resistant to whitefly.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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