

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

**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 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.

**INTRODUCTION**

31 Cotton (*Gossypiumhirsutum*) is the world's chief natural fibre crop. It is grown in the areas  
32 of temperate and tropical climates. Cotton utilization is expanding, compared to a significant  
33 expansion in population throughout the planet. Pakistan is a farming nation, and cotton is  
34 the second most significant crop contributing a considerable part to the economy. About 1.3  
35 million farmers (out of 5 million) foster cotton on a range of 3 million hectares, covering  
36 15% of the cultivated zone in the country. Cotton and cotton goods account for 1.6% of the  
37 GDP and 55 % of the foreign exchange incomes [1]. Cotton is also named as "White Gold"  
38 due to its importance in different sectors. Pakistan had a glorious past in terms of cotton  
39 production, now facing a decline in production over the years.

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

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

60 In order to improve our cotton crop against the whitefly, natural variability and divergence  
61 between crops should be broadly identified and distinguished [7]. Studying genetic diversity

62 can help us find the supply of many novel traits presenting tolerance to different biotic and  
63 abiotic stresses like whitefly. Diverse lines are required for defect correction of commercial  
64 varieties and the establishment of novel varieties. So, identification of diverse lines (if  
65 available), formation of diversity (if not available or limited), and its resulting usage are the  
66 major areas of any yield improvement programs. Genetic diversity within and between crop  
67 plant species allows the breeders to choose superior genotypes to be directly utilized as a  
68 new variety or as a parent in a hybridization program.

69 Different methods are available for genetic diversity analysis. Diverse DNA markers for  
70 insect genetics research (i.e., the amplified fragment length polymorphism (AFLP) marker,  
71 expressed sequence tags (EST), mitochondrial DNA, microsatellites, and random amplified  
72 polymorphic DNA (RAPD) were diagnosed and advanced to decide the populace genetic  
73 shape of a species [8]. In this research, we will use microsatellite markers. These markers  
74 will be utilized in the cotton improvement for broadening the genetic base and developing  
75 varieties against pests and diseases. Microsatellites are particularly famous genetic markers  
76 due to their co-dominance, excessive plentiful variant and polymorphism rates, more than  
77 one allele, and short allele detection through many methods [9]. Microsatellite markers are  
78 also potent in population genetic research for insect species. Through molecular genetic  
79 prognosis and the use of populace genetic analyses, powerful manipulation may be carried  
80 out quickly at a low cost. Various current researchers have hired different microsatellite  
81 markers to find the populace genetic shape, genetic differentiation, genetic evolution, gene  
82 flow, and dispersal sample of *B. tabaci* over extraordinarily massive geographic scales [10].

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

92 current research focuses on identifying white fly resistant cultivars for improved cotton  
93 yield.

94

## 95 **MATERIALS AND METHODS**

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

100 **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

101

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

## 105 **DNA extraction, quantification, and PAGE**

106 DNA was extracted from young cotton leaves by the cetyltrimethyl ammonium bromide  
107 (CTAB) extraction method (Doyle and Doyle, 1987) with a few modifications [12]. 2µl of the

108 DNA sample from each cotton genotype was resolved on 1% agarose gel check the DNA  
109 samples' quality and quantity [13].

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

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

116

## 117 RESULTS

118 In order to analyze the genetic diversity of 50 *G. hirsutum* successions, ten Simple sequence  
119 repeat (SSR) marker pairs were employed. By utilizing these ten primers, a sum of 58 loci  
120 were established. The highest range of loci was 8. Eight bands were amplified by three  
121 markers that were NAU-883, NAU-2714, and BNL-827. The lowest range of loci was 2. Two  
122 bands were amplified by one marker, which was JESPER-101. Regarding genetic diversity, the  
123 marker that showed maximum genetic diversity was NAU 2161, with a value of 0.8920.  
124 0.4136 was the minimum value of genetic diversity given by the marker BNL 1672. 0.7422  
125 was the mean value of genetic diversity which ranges between 0.8920 and 0.4136 (Table-2)

126 **Table2 :Gene diversity and PIC calculation**

Marker	Major Frequency	Allele	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

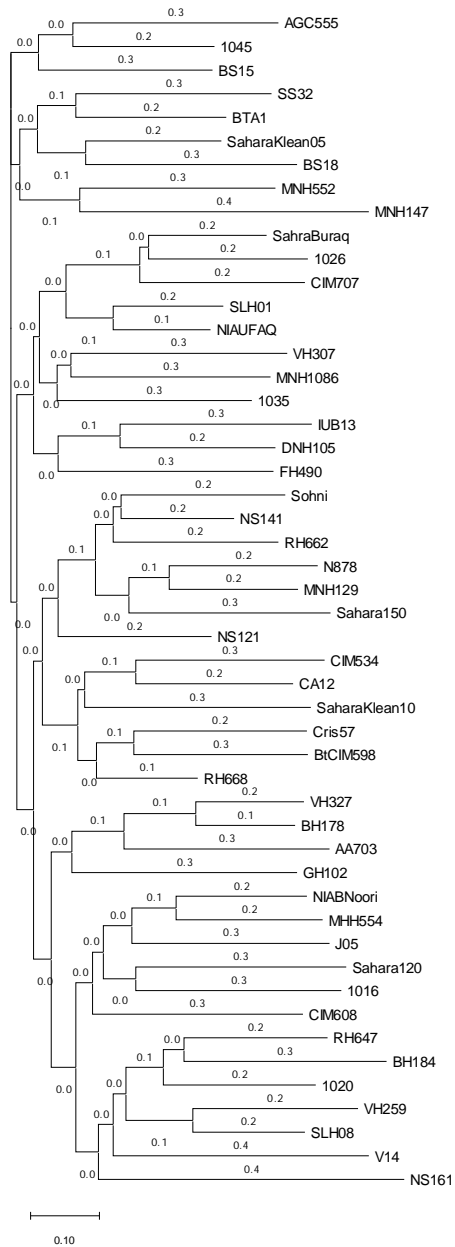
127

## 128 **Similarity Index**

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

## 132 **Phylogenetic tree**

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



139

140 **Fig-1: Dendrogram of 50 cotton varieties**

141 **DISCUSSION**

142 The genetic diversity of 50 cotton varieties was explored using 10 Microsatellite markers.  
 143 These markers formed 58 loci. The NAU-2161 marker expressed the most significant level of  
 144 polymorphism. In contrast, the BNL-1672 marker exhibited its lowest level. The maximal  
 145 value of allele number was eight, and it was exhibited by NAU-883, NAU-2714, and BNL-827  
 146 markers. 2 was the minimal value of allele number exhibited by JESPER-101. Addressing

147 about genetic diversity, the highest value of 0.8920 was revealed by the NAU-2161 marker.  
148 BNL 827, NAU 2083, and NAU 883 also displayed close maximum genetic diversity estimates  
149 of 0.8544, 0.8312, and 0.8256, respectively. The most negligible value of genetic diversity  
150 was portrayed by BNL-1672, which was 0.4136. The range of genetic diversity is between  
151 0.8920 and 0.41360, with a mean value of 0.7422. To figure out the polymorphism of all 10  
152 SSR markers, polymorphism information content (PIC) analysis was applied. NAU 2161 gave  
153 a maximal polymorphism standard of 0.8828. BNL 1672 can be seen to give a minimal  
154 standard of polymorphism that was 0.4034. The range of genetic diversity comes between  
155 0.8828 and 0.4034, with a mean value of 0.7215.

156 Some cotton varieties show maximum similarity, and some show minimum similarity.  
157 Genetic diversity is maximum in varieties NS-161, VH-307, and AGC-555 as they are located  
158 at the most significant distance in clusters. The maximal value of genetic distance was 1.00,  
159 and the minimum value was 0.5 base pairs. The minimum relation was found between BH-  
160 178 and AA-703, AGC-555 and 1045, Cris-57, and Bt CIM 598, respectively. The maximum  
161 relation was found between wide varieties, including BH-184 and 1045, BS-18 and BH-178,  
162 DNH105 and NS-161, respectively. Polymorphic SSRs can be highly informative for molecular  
163 genetic diversity studies in various cotton varieties [16].

164

165

## 166 CONCLUSION

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

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