Original Research Article

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

ABSTRACT

Cotton plays vital part in agriculture sector ultimately entire economy. Owing to lesser genetic diversity, cotton harvest is being influenced by various biotic and abiotic factors. Amongst biotic factor, whitefly is a main factor. Obliteration of cotton crop on large level by the whiteflies was the prime factor in the verdict to develop novel methods for cotton breeding programs. Genetic diversity of crop plants is the basis for the sustainable development of novel varieties. So, we aim to characterize the diverse genetic resources utilizing various statistical tools and then use them in the breeding program. Our objective was to identify whitefly resistant cotton varieties that will improve the quality, yield and growth of cotton crop. In this study, we used 10 Microsatellite (SSR) markers for genetic diversity assessment among 50 genotypes of G. hirsutum. The primers used were of series NAU and BNL. 58 loci were founded by using these markers. Highest range of loci was 8. Eight bands were amplified by 3 markers that were NAU-883, NAU-2714 and BNL-827. Lowest range of loci was 2. Two bands were amplified by 1 marker that was JESPER-101. The mean value of PIC was 0.7215 which ranges between 0.8828 and 0.4034. NAU 2161 displayed maximum polymorphism value of 0.8828 and BNL 1672 showed minimum value of polymorphism value of 0.4034. Cluster analysis was also conducted. It showed that there are 4 clusters in which 50 genotypes were grouped. Cluster A hold 30 varieties. Cluster B include 11 varieties. Cluster C had 6 varieties and cluster D had 3. Genetic diversity is maximum in varieties NS-161, VH-307 and AGC-555 as they are located on greatest distance in clusters. The SSR genetic profile found for every one of the cultivars made it conceivable to separate a few cultivars. To conclude, this investigation of the genetic divergence of cotton cultivars with SSR markers support 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 hereditary variety of cotton cultivars to fulfill the need of cotton development for various purposes.

KEYWORDS: Whitefly, SSR Markers, Cotton plant, Resistance.

INTRODUCTION

Cotton is the world's chief natural fibre crop. It is grown in the areas of temperate and tropical climate. Cotton utilization is expanding, comparing to a great expansion in population all throughout the planet. Pakistan is a farming nation, and cotton is the second most significant crop contributing a huge part in 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 reason 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 an immense age of cotton cultivation in the past that has deteriorated to an enormous level over the years. Now only 60 percent of foreign exchange is attained from cotton.

The variables liable for low yield incorporates lack of endorsed seed, pest attack 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 are dreading a loss in their cotton crop due to high temperature, rains and attack of whitefly [2]. The production estimate of cotton crop has been reduced to 7.4 million bales. The whitefly, *Bemisia tabaci* is a dangerous pest of numerous vegetables, ornamented and farming harvests in tropical and subtropical nations of the world [3]. It consumes food from an average of 900 different host plants, including species of financial significance related to the 63 families. Additionally, it also transfers more than 111 plant infections including cotton leaf curl infection (CLCuD) in American cotton [4]. Sucking pests and lepidopteran caterpillar can invade the cotton though out their development cycle. After applying insecticide whitefly populace arises back shortly as eggs and nymph swell at foliage's basement and on the lower region of leaves. Sugary material called as honey dew secreted by the whitefly, draws the sap of cell and the area for photosynthesis is decreased because of which a black sooty mould happens on the leaves that diminishes the yield, value and also the importance

of crop [5]. The attack of *B. tabaci* in cotton has become hard to control with insect sprays in light of the fact that whitefly lives on the underside of the leaves and its more limited formative period which makes them resistant to many insecticides (Organophosphate, Carbamate and Neonicotinoids) and resistant strains have gotten increasingly bountiful [6].

Diversity can be defined as the level of differentiation between or inside species. In order to improve our cotton crop, 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 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. Occurrence of genetic diversity within and between crop plant species allows the breeders to choose superior genotypes so that they can be directly utilized as new variety or as parent in 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 as well as the development of 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 a huge form of methods [9]. Microsatellite markers also are very powerful gear in populace genetic research for insect species. Through molecular genetic prognosis the use of populace genetic analyses, powerful manipulate may be carried out in a brief time at a low cost. Different microsatellite markers had been hired in numerous current researches to find the populace genetic shape, genetic differentiation, genetic evolution, gene flow, and dispersal sample of *B. tabaci* over extraordinarily huge geographic scales [10].

Microsatellite markers can be used for aiding in determining the nature and degree of genetic diversity among inbred lines. It can also be helpful for appointing inbred lines

effectively to heterotic sets and creating the decision of heterotic parents 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 totally on microsatellite markers is blended with environmental approaches, the development of an effective framework for dealing with *B. tabaci* is facilitated. Thus, based on above facts, current research is being focused on identification of white fly resistant cultivars for improved cotton yield.

MATERIALS AND METHODS

Fifty genotypes of cotton were grown in Cotton Research Institute of Multan. Their names are listed below.

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

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

DNA extraction, quantification and PAGE

For good DNA extraction, it is best suited that young and healthy leaves should be collected. DNA was extracted from cotton young leaves by cetyl trimethyl ammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987) with a few modifications [12]. For DNA quantification, agarose gel electrophoresis method was used to quantify DNA in order to determine how much DNA we have and to see if our DNA is intact or in accurate size. Mullis invented PCR in 1986 for amplification of DNA samples [13]. To find out the diversity microsatellite marker was 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.

Statistical tools for genetic diversity analysis used just as Cluster analysis and used Softwares for genetic diversity analysis.the used data in population genetics analyses, power marker software.

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 10 primers, a sum of 58 loci were established. Highest range of loci was 8. Eight bands were amplified by 3 markers that were NAU-883, NAU-2714 and BNL-827. Lowest range of loci was 2. Two bands were amplified by 1 marker that was JESPER-101. Talking about genetic diversity, the marker that showed maximum genetic diversity was NAU 2161 with the value of 0.8920. BNL 827, NAU 2083 and NAU 883 also gave near to maximum genetic diversity values that were 0.8544, 0.8312, 0.8256 respectively. 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

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 minimal value of genetic distance was 0.5.

Phylogenetic tree

A phylogenetic tree, otherwise called a phylogeny, is an illustration that portrays the lines of developmental lineage of various varieties from a common predecessor. Software like power marker was operated to build phylogenetic tree. Varieties was made into cluster form by the phylogenetic tree. These clusters were further divided into sub clusters and sub sub-clusters of entire clusters. The main clusters that were developed were four named as A, B, C and D. These main clusters then undergo more division into sub clusters and sub sub-clusters. Cluster A hold 30 varieties. Cluster B include 11 varieties. Cluster C had 6 varieties and cluster D had 3 (Fig-1)

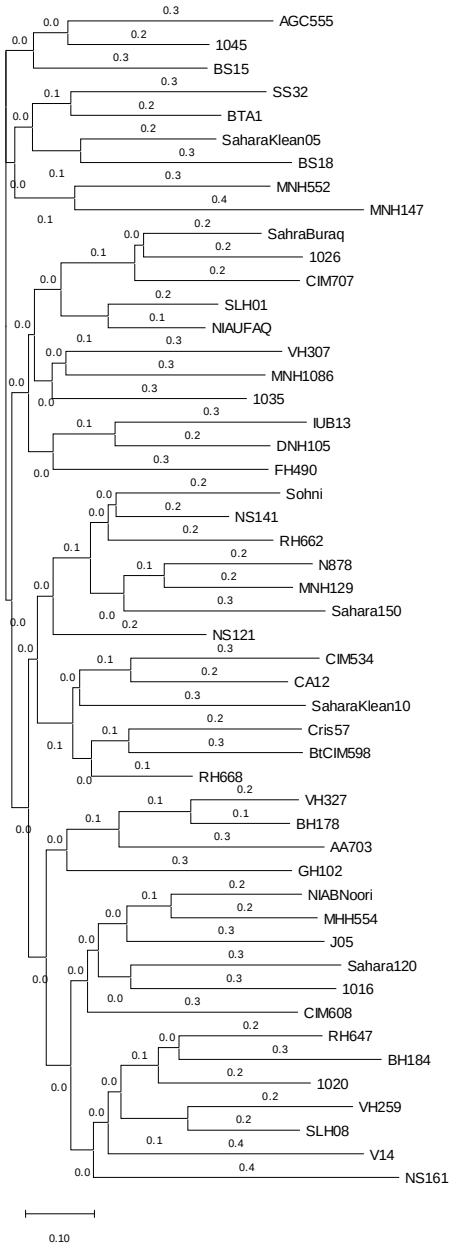

Fig-1: Dendrogram of 50 cotton varieties

DISCUSSION

Genetic Diversity of 50 cotton varieties were genotyped by using 10 Microsatellite markers. These markers formed 58 loci. The greatest level of polymorphism was expressed by NAU-2161 marker. While its lowest level was exhibited by the BNL-1672 marker. 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 NAU-2161 marker. BNL 827, NAU 2083 and NAU 883 also displayed close by maximum genetic diversity estimates that were 0.8544, 0.8312, 0.8256 respectively. The least 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 its mean value of 0.7422. To figure polymorphism of all 10 SSR markers, polymorphism information content (PIC) analysis was applied. NAU 2161 gave maximal polymorphism standard of 0.8828. BNL 1672 can be seen to give minimal standard of polymorphism that was 0.4034. The range of genetic diversity comes between 0.8828 and 0.4034 with the mean value of 0.7215.

To analyze the data obtained from SSR markers, 1973 Nei's coefficient-based software was used. 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 on greatest 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. The maximum relation was found between many varieties. Some of which include BH-184 and 1045, BS-18 and BH-178, DNH105 and NS-161. Some articles showed polymorphic SSRs can be highly informative for molecular genetic diversity studies in various cotton varieties [14]. The relationship found by using SSRs have also been mentioned in many other papers.

CONCLUSION

Hereditary variety has now been recognized as a particular region that can contribute in food and wholesome security. Better comprehension of hereditary variety will help in figuring out what to moderate as well as where to save. Hereditary variety of harvest plants is the establishment for the feasible advancement of new assortments. So, there is a need to describe the assorted hereditary assets utilizing different measurable devices and use them in the rearing project. With the appearance of high throughput atomic marker innovations, it is feasible to portray bigger number of germplasms with restricted time and assets. The examination depends on measurable instruments for better understanding. The variety demonstrated by various examination can additionally be used in heterosis reproducing, rearing.

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