

Evaluation of genetic potential of cotton lines against whitefly tolerance

ABSTRACT

Cotton (*Gossypium hirsutum* L.) is considered as main source of natural fiber, fuel, wood and oil all over the world and essential source of raw material for the textile industry. Substantial loss of cotton is due to a variety of pests and insects. White fly (*Bemisia tabaci*) is one of the pest which attacks the cotton with many bio types and is distribute worldwide and has broad genetic diversity. Few groups within *Bemisia tabaci* are important pests of a range of agricultural, horticultural and ornamental crops throughout the world. In this research work, the genetic diversity of cotton and examine the high level of resistance in the genotype of cotton that serve as a basis for their genetic improvement against whitefly tolerance was studied. Through the genetic potential of cotton was analyzed genotypically and wide sense heritability, as these are common approaches use to detect association and inheritance of the target and component traits. SSR primer (NAU 988) showed highest level of polymorphism was 0.96. But lowest level of polymorphism was detected in (NAU 5121) with 0.36 PIC value. Average value of Polymorphism information content (PIC) calculated for overall observation was 0.73. Pair wise genetic estimation based on Nei 1973 ranged from 0.500 to 1.00. Bootstrap neighbor joining (NJ) based UPGMA (Unweighted pair group method with arithmetic) dendrogram were as follow: main six clusters are formed mentioned as A, B, C, D, E, and F. maximum accession falls in a single cluster representing low genetic diversity. Low genetic diversity was observed in genotype as maximum accession share the same group. The upland cotton accessions with far distance from each other such as FH 326, SLH 07, FH18 and Cris 541 are greatly suggested to breeder for breeding and develop new cotton lines. If theses cotton lines variant from the previous, have more chance of resistance against whitefly attack. This also commend explaining the genetic diversity by utilization of SSR primers to advance the resolution of upland cotton cultivars for worthy breeding programs.

Keywords: Cotton, Whitefly, SSR, Diversity, Resistance

INTRODUCTION

Generally, name of cotton derived from Arabic word "Quotn"[1]. Cotton crop (*Gossypium hirsutum*) has multiple uses, belongs to family name as Malvaceae and the name of its

genus is *Gossypium* [2]. Cotton have 8 diploid genome arranged as A to G and 1 allopolyploid genome named as (AD). Out of 50 species of cotton crop only 4 species are cultivated, name as *Gossypium hirsutum*, *Gossypium barbadense* are tetraploid *Gossypium arboreum* and *Gossypium herbaceum* are diploid. *Gossypium hirsutum* and *Gossypium barbadense* also called American cotton. *Gossypium arboreum* and *Gossypium herbaceum* also called Asian cotton. Phylogenetic study shows *G. hirsutum* AD was result of hybridization of 2 diploid species named as *G. arboreum*(A) and *G. raimondii* (D) [3]. The number of chromosomes in cotton crop are 13. There are fifty species of cotton crop, out of them forty five are diploid as $2n= 26$ and five species are tetraploid $2n= 52$ that are reported. Pakistan is rank fifth producer and 3rd main exporter of cotton crop [4]. India ranked 1st, China ranked 2nd, USA ranked 3rd throughout the world [5]. Brazil ranked 4th over all the world [6]. In Pakistan cotton industry facing several problems during trading at international level such as competition for synthetic fiber, deprived fiber quality and stumpy revenue primarily due to outbreak of numerous lethal microbes. The crops of cotton are attacked by thousands of dangerous pathogens that because severe diseases are divided in to two categories such as sucking and eating microbes. First, sucking microbes damaged the plants through sucking essential nutrient of plant and made them flabby, yellowing, drying plant and fall fiber quality and also act as vector for various viruses specifically white fly. Second, eating microbes directly eat the vegetative part of cotton crop. It is estimated mostly cotton yield decline due to violence of sucking and eating microbes [7]. About 11,00 reported disease of plants are caused by viruses, more than 30% DNA viruses are transferred through pest especially white fly. Lethal viruses that cause disease in plants normally start 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 disturb growth and transportation are poor understood[1]. white fly (*Bemisia tabaci*) belongs to *Aleyrodidae* and its genus name is *hemipteran*. Whiteflies are complex species with 34 distinct species, 392 holotypes, 44 cryptic species and 24 altered biotypes. It is facultative endosymbiont. Whitefly imbibe phloem juice from cotton plant and excrete honeydew like sticky liquid on the surface of cotton leafs and bolls. Whitefly act as vector for plant viruses and DNA viruses name as *Begomoviruses* that is single stranded which leads cotton leaf curl disease in upland cotton cultivars. White fly has great reproductive potential, minute size, wide diversity, board host range and compliance. Due to their characteristics it shows

great tolerance against insecticides such as pyrethroids, organophosphates, acephate and neonicotinoid which are used for white fly management [8]. The most commonly utilized classical methods to check insect potential and application of insecticides. Various traditional and biochemical approaches such as integrated pest management (IPM), biological control that produced parasitoids. However, pathogenic fungi are used that produce mycoinsecticide to control whitefly attack. Yet, because of their rapid reproductive potential, they can easily stunned the cotton crop provoking breeders used effective doses of insecticides and pesticides when the amount of flies per leaf is few [9]

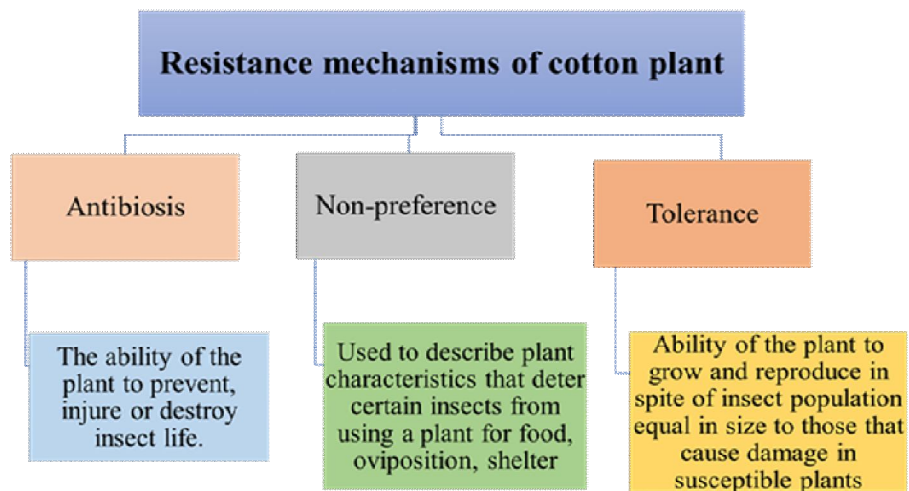


Figure 1: Resistance mechanisms of cotton plant

Plants have naturally occurring resistance mechanism to fight against pathogens name as antibiosis, tolerance and non-preference that work in relation to pathogen attack. In various studies showed, induced tolerance in DNA sequence of cotton crop that are attack by white fly. Sometimes, antibiosis and antixenosis work mutually against white fly attack [10]. Molecular marker such as SSR, RAPD, RAFLP, SNP and next generation sequencing(NGS) used to improve cotton varieties against white fly tolerance. Genome wide association study (GWAS) has been used in cotton cultivar for evaluation of genetic diversity and association mapping that ultimate result better quality and quantity of cotton fibers [11]. Based on above this research is focused on identifying cotton lines having tolerance against white fly.

MATERIALS AND METHODS

Collection of samples

Samples of cotton seed were acquired from Central Cotton Research Institute (CCRI) Multan Pakistan and was grown in Randomized Complete Block design. Fresh Leaf of 50 cotton accessions were collected (Table 1) for DNA isolation in zipper lock plastic bags and labelled with black marker. These plastic bags were positioned in ice box to protect leaf samples from sunlight during travelling. These leaf samples were stored at -20°C until DNA extraction was started. Experimental work carried out in laboratory of Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan.

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	36	GH 99
16	FH 118	33	Cris 628	49	VH 282
17	FH 142	34	Cris 635	49	VH 282

DNA Extraction

To study the genome of cotton, isolation of DNA was preliminary phase. DNA from specific leaflets of cotton accession was isolated through the use of CTAB method [12] with narrow modifications

DNA Quantification

To confirm the quality and quantity of extracted DNA was assessed by loading extracted DNA along with DNA ladder in electrophoresis system via agarose gel electrophoresis. After confirmation isolated DNA stored at -20°C. DNA quantification was performed through 0.8% agarose

SSR primers analysis

SSR primers were chosen casually and it enclosed most of the genome of cotton crop. SSR primers selected because of their codominance, multi allelic, widely spread on whole genome of cotton crop and showed high PIC value than RAPDs primers. Twenty SSR primer pairs used in this research work. These 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].

Polymerase chain reaction

PCR was performed after DNA quantification. Volume of reaction mixture for PCR was 20ul consisting on DNA sample (3ul), master mix (10ul), forward, reverse primers (1.5ul, 1.5ul), MgCl₂ (0.5ul) and PCR water (2.5ul). Particular SSR primers was carefully chosen for specific DNA segment which were liable for whitefly tolerance. The PCR amplification profile was consisting 1 cycle of initial denaturation at 94°C for 7: 00 min, along with 35 cycles of denaturation at 94°C for 1:00 min, annealing at 55°C for 1:00 min and extension at 72°C for

1:00 min followed as 1 cycle of final denaturation at 72°C for 7:00 min. the last step of PCR was storage at 4°C for infinite time. PCR was carried out for all extracted DNA of 50 chosen cotton accessions by using 20 SSR primer pairs.

Polyacrylamide gel Electrophoresis

PAGE was done after PCR to check the position of PCR products on gel. Generally, it was used separate DNA bands in kind of 1-100bp, based on DNA quantity loaded in vertical system of electrophoresis. Liquid mixture of gel consisted on acrylamide solution (11.25ml), ammonium per sulfate (400ul), 1X buffer (26.25ml) and TEMED (30ul). Ammonium per sulfate and TEMED pour at a time it was helpful for polymerization of gel. Solution of liquid was poured in glass plates. Electrophoresis was done in 1X buffer at 120v and 70Amp for 1 hour. At the end bands of DNA was observed by staining with silver nitrates and seen under illuminator (Fig-2).

Data analysis

To relate the 50 varieties of cotton crop were accomplished based on perfect existence or lack of DNA bands prepared by 20 SSR primer pairs. These DNA bands evident on polyacrylamide gel electrophoresis were kept scored manually "1" identifying the existence of allele and "0" demonstrating nonexistence of allele. Only clearly visible bands were scored and polymorphism was checked on the base of their presence and absence. An important software named as power marker v 3.25 was used to check genetic diversity, allele number, major allele frequency, genetic distance and PIC of 50 accessions of cotton crop.

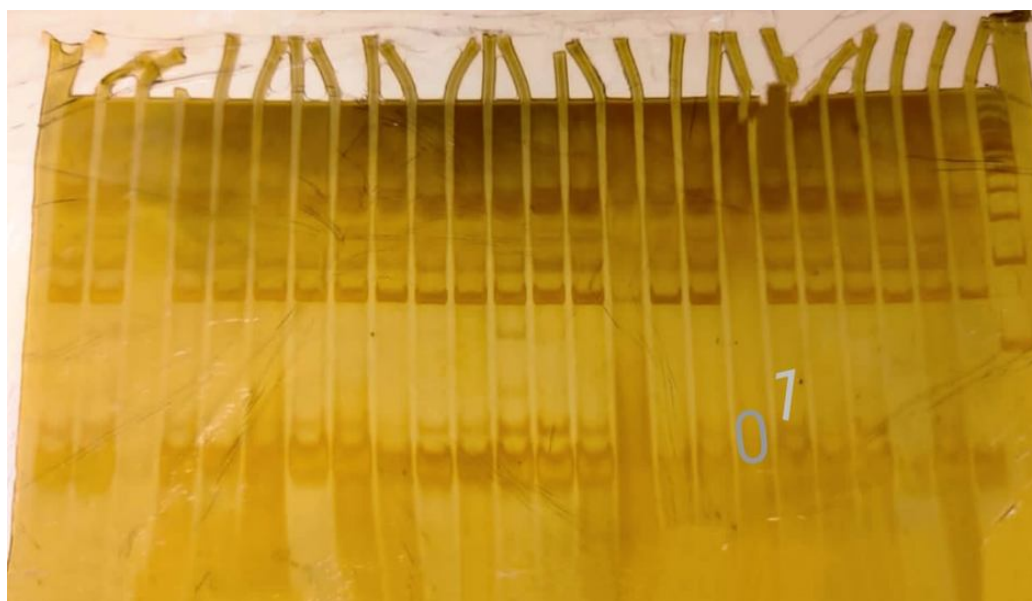


Fig- 2: PAGE gel and its scoring

RESULTS

Estimation of allele numbers

Twenty SSR primer pairs were used to check genetic diversity among 50 cotton genotypes. Total no. of 102 loci which were amplified by 20 SSR primers average loci per each primer was 1.5. the number of loci for each maker arranged from 1 to 8. Maximum number of allele 8 amplified by SSR primer pairs name as NAU2083, NAU 883 and NAU 988. While minimum number of allele 2 amplified by SSR primers name as NAU 5121 and BNL 2443 (Table-2).

Table 2: Primer, position of primers and their allele number

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			

Assessment of allele number, Genetic diversity and polymorphism information content (PIC) value

An important software power maker v. 325 used to check genetic diversity among 50 cotton genotypes. Average value of genetic diversity was 0.75 which were settled 0.39 to 0.96. maximum level of genetic diversity observed in NAU 988 while minimum level of genetic diversity shown by NAU 5121.

To assess polymorphism level by calculating PIC value with utilization of 20 SSR primers among 50 cotton accessions. Maximum level of polymorphism shown by NAU 988, its PIC value was 0.96. while low level of polymorphism 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 SSR primers were polymorphic but some of them was monomorphic (Table-3).

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

Frequency based pair wise similarity

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

Table 4: frequency based pair wise similarity among 50 accession

OTU	B 021	Barhi M1	BtCIM599	Chandni 95	CIM 496	CIM 506	CIM 554	CIM 573	CIM 591
B 021	0.0000								
Barhi M1	0.7000	0.0000							
Bt CIM 599	0.7500	0.9000	0.0000						
Chandni 95	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 341	0.6500	0.9500	0.7000	0.7000	0.9000	0.9000	1.0000	0.7000	0.7000
Cris 362	0.8500	0.6000	0.8500	0.8000	0.7500	0.9000	0.7500	0.8000	0.6500
Cris 380	0.6500	0.8500	0.8000	0.7000	0.9500	0.8500	0.9500	0.7500	0.6000
Cris 383	0.7000	0.8000	0.7500	0.7500	0.8500	0.7500	0.8500	0.7500	0.8000
Cris 387	0.8000	0.6500	0.9500	0.8500	0.8000	0.9500	0.8500	0.8500	0.7000
Cris 390	0.8500	0.7500	0.8000	0.7500	0.8000	0.7500	0.7000	0.8500	0.8000
Cris 399	0.8000	0.7500	0.8000	0.8000	0.8500	0.8000	0.6000	0.9000	0.9500
Cris 401	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
CRS2A 3R	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
FH Lalazzar	0.9500	0.7000	0.6000	0.8000	0.9000	0.7500	0.8500	0.8500	0.7500
FH 114	0.8500	0.6000	0.7000	0.8500	0.9500	0.8500	0.8500	0.7500	0.6000
FH 118	0.8500	0.7000	0.7000	0.9500	0.9000	0.8500	0.9000	0.6500	0.6500
FH 142	0.7000	0.7500	0.8500	0.7500	0.8500	0.8500	0.8000	0.7500	0.8000
FH 152	0.9000	0.7500	0.8500	0.8000	0.8500	0.7500	0.8500	0.9000	0.8500
FH 326	0.6000	0.8000	0.5000	0.6500	0.8500	0.9000	0.8000	0.5500	0.7000
FH 941	0.8000	0.8000	0.7000	0.7000	0.9500	0.8000	0.9000	0.7500	0.8000
FH 942	0.9000	0.8500	0.7000	0.8500	1.0000	0.9000	0.9500	0.7000	0.7500
GH 114	0.9000	0.8000	0.7500	0.8000	0.9500	0.7000	0.8500	0.8500	0.9000
GH 99	0.8000	0.7500	0.7000	0.8000	0.9500	0.8000	0.7500	0.7500	0.9000
Gornal 93	0.9000	0.7500	0.9000	0.7000	0.9000	0.8000	0.8500	0.9000	0.9000
Harli 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 50	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
SH 06	0.7000	0.7500	0.8000	0.7500	0.9500	0.8000	0.7500	0.7000	0.8000
Sindh 01	0.8500	0.7500	0.8500	0.9000	0.9500	0.7000	0.8000	0.7000	0.9000
SIM 04	0.7500	0.7500	0.7500	0.8500	0.8500	0.8000	0.7500	0.7500	0.8000
SIM 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

Phylogenic tree

Phylogenic tree was constructed through bootstrap neighbor joining (NJ) technique based on Nei 1973 method. Fifty cotton genotypes divided into clusters based on their similarity coefficient. The UPGMA dendrogram made six main clusters named as A, B, C, D, E and F. UPGMA is abbreviation of unweighted pair group method with arithmetic mean. These 6 main clusters also divided into sub group, sub- sub group The Cluster A, B, C, D, E and F contain 27,10, 6, 5, 1 and 1 accessions respectively (Fig-3).

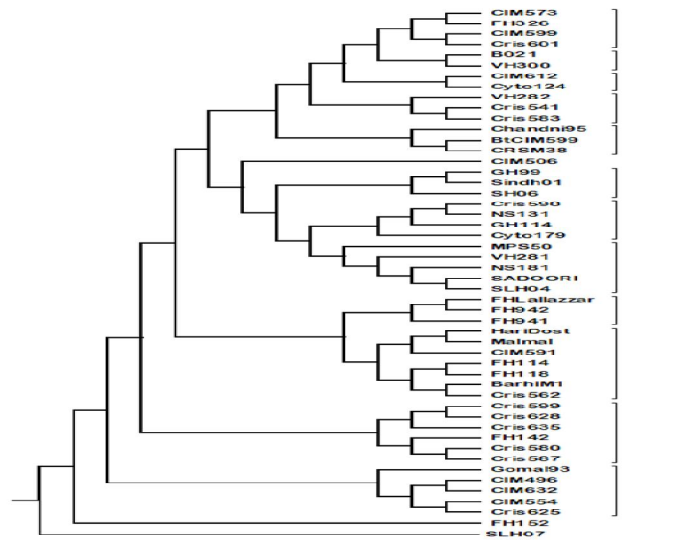


Fig-3: Triangular form of UPGMA Dendrogram displayed genetic relationship among 50 accession

DISCUSSION

Evaluation of genetic diversity amongst cotton cultivars provide important information that was helpful in the development of diversity and conserve cotton crop. Molecular markers used to evaluate genetic diversity and in improvement of cotton crop, because these marker have segment of gene containing useful traits [16]. In this research work, we focused on effectiveness of SSR markers among 50 accessions of cotton crop. SSR markers were used because of their abilities such as multi allelic, not required pure template DNA, hypervariable nature even among closely related varieties shown allelic variation, easily and automatically scored. In this research work, twenty simple sequence repeat (SSR) primers were used to evaluate genetic diversity between fifty cotton genotypes. Out of twenty 80% SSR primers were polymorphic while 20% were monomorphic. The aggregate number of allele amplified by these SSR markers was 102 and the average value of allele for each primer was 1.5 that were 73% mutually informative. The total no. of loci for each marker were arranged (1-8). NAU 2083, NAU 833 and NAU 988 these SSR markers displayed 8 bands in the research work. SSR marker named as NAU 988 showed highest level of

polymorphism, because it displayed 96% polymorphism. In addition, some of them also showed high level of polymorphism names as NAU 1070, JESPER 274 and JESPER 153, their PIC values ordered as 91%, 90% and 85%. But 2 SSR markers named as NAU5121 and BNL 2443 were showed 39% and 43% polymorphism. On the other hand, none of them SSR primers separate overall cotton genotypes. Five SSR primers showed high gene diversity named as JESPER 274, NAU 1070, JESPER 153, BNL 3651 and BNL 134, 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 the used of valuable software power marker v 3.25 and [17] method. this method is used to find similarity matrix value that produced high degree of association among 50 cotton genotypes. Level of pair wise similarity was arranged as 0.50 to 1.00. highest level of pair wise similarity was observed in cotton genotypes named as B-021, V 300, CIM591 and Malmal. While lowest level of pair wise similarity was observed in cotton genotypes named as CIM554, Cris541, CRMS38 and CIM496. UPGMA dendrogram was constructed by using bootstrap neighbor joining (NJ) technique that was based on important method named as [17]. On the base of 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 also distributed into small groups, sun group and sub-sub groups. Cotton genotypes named as CIM 496, CIM632, CIM 554 and Cris 625 showed high genetic relation and found in same cluster. On the other hand, CIM 506, MPS 50 and VH 282 share the same group.

CONCLUSION

We should not only rely to chemical means for managing white fly control. Learned and studied genetic diversity were help us to preserve genetic information of white fly resistant cotton varieties for better cultivation in future. Under changing environmental conditions, evaluation of genetic diversity played a vital role in the starting of any breeding plans especially for cotton crop. In our study, PIC value and pair wise similarity were calculated, constructed UPGMA phylogenic tree to check genetic diversity among 50 cotton genotypes. Most of the genotypes showed low genetic diversity because they fall in same group. While other displayed great genetic diversity because they exist in diverse group. Our results revealed cotton genotypes named as FH 326, SH 07, FH 18 and Cris 541 have great genetic diversity. therefore, these cotton genotypes preferred for next breeding and develop new

lines of cotton crop. When these lines different from earlier showed great chance of tolerance against white fly outbreak.

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