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Research Article

TRACING THE DIVERSITY OF CULTIVATED TRANSGENIC COTTON IN PAKISTAN BY SSR BASED DNA FINGERPRINTING

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Abstract

The introduction of transgenic *Bacillus thuringiensis (Bt)* cotton is one of the success stories of applying biotechnology in agriculture. It also decreased the use of insecticides and the production cost as an environmental and economic benefit, respectively. The use of selective genotypes as a parental material for *Bt* cotton breeding has resulted in the multiplication of limited germplasm. The main objective of the study was to find out the genetic diversity of cultivated *Bt* cotton genotypes by fingerprinting. Twenty-two SSR markers were used to analyze the genetic differences in *Bt* cotton genotypes, collected from two different sources i.e., research institutes and open market. Results showed that the Polymorphism information content (PIC) value ranged from zero to 0.673. A UPGMA based dendrogram divided all the genotypes into four main clusters. Eighty-six percent similarity was observed between VH-282 and AS-01 while the lowest similarity of 45% was found between IUB-222 and FH-167.This diversity of newly introduced transgenic genotypes will help to detect the diverse lines to be used in future for broadening the genetic base of *Bt* cotton in Pakistan. Another observation was that a genotype, FH-114 o*Bt*ained from two sources was clustered in two different clades. This showed the presence of name variants for a particular genotype depicting adulteration of seed available in seed market which need to be addressed. Though the adoption of *Bt* cotton was rapid, but the economic impact of this adoption was not significant in Pakistan as compared to other developing countries. The identified problem might be one of the reasons, limiting the benefits of this technology to be harvested out of it.

Keywords: *Bt* cotton, Genetic diversity, SSR markers, UPGMA based dendrogram
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1. INTRODUCTION

Cotton (Gossypium *hirsutum* L.) crop has the longest value chain due to which contribute significantly to the rural economy of the country. Raw cotton bales and textile products has a major share in total exports of the country. In the year 2022-2023, cotton crop contributed 0.3 % to GDP and 1.4 % in value addition in agriculture (GOP, 2023). Cotton crop was at stake before the introduction of transgenic *Bacillus thuringiensis* (*Bt*) cotton due to heavy pest infestation and the indiscriminate use of costly pesticides (Ahsan and Zafar, 2009). The *Bt* cotton was introduced in Pakistan during 2002 and is grown in more than 90 % area of cotton in Pakistan (Marral et al., 2023). *Bt* cotton is proved to be eco-friendly and beneficial as it has reduced the insecticide usage (Kumar et al., 2008; Tokel et al., 2022).

For the development of transgenic cotton, gene transformation is done in tissue culture responsive genotypes and then transferred to local elite varieties by conventional breeding (Ullah et al., 2012) Pakistani *Bt* varieties were produced by backcrossing the local germplasm with exotic transgenic cotton. Therefore, utilization of few selective local varieties as a recurrent parent in the breeding programme has resulted in genetic similarities among the developed *Bt*

varieties (Ullah et al., 2012), which is not desirable for sustainable cotton production. Molecular breeding techniques along with traditional phenotypic selection are being utilized efficiently to improve various traits in cotton (Cantrell, 2008). DNA markers provide a valuable tool to study relationship between cultivated and wild species and to study genetic diversity in crop germplasms (Bertini et al., 2006). As well as they have a great potential to find genetic similarities between two or more genotypes. Several molecular markers were used to characterize the cotton such as random amplified polymorphic DNA (RAPD) (Iqbal et al., 1997), restriction fragment length polymorphism (RFLP) (Wendel and Brubaker, 1993),, amplified fragment length polymorphism (AFLP) (Rana et al., 2005) and simple sequence repeat (SSR) markers (Reddy et al., 2001; Lacape et al., 2007). Nowadays, there is large number of SSR markers (CMD website, http://www.cottonmarker.org) available for the characterization of cotton.

In cotton, SSRs represent a unique type of DNA markers, which are also called microsatellites. These are the short tandem repeats containing two to six nucleotide units and are highly abundant in eukaryotic organisms. DNA fingerprinting or traceability of genetic diversity and relatedness of cotton genotypes is very useful for germplasm evaluation. Due to their high reproducibility, co-dominant and multiallelic in nature, SSR markers, has been widely used for DNA fingerprinting in different crop species as compared to other DNA markers (Yu et al., 2012).

It was not until 2010 that *Bt* cotton was officially approved for commercialization for the first time in Pakistan. But the unapproved seeds of the *Bt* cotton have been found in the market since early 2000s without any stewardship (Ali and Abdulai, 2010). So, this situation has raised the distribution mixed/adulterated seed in the cotton seed market under the label of *Bt* cotton seed. The cotton seed market in Pakistan is still in disarray for the quality seed production. Therefore, the framers do not know the actual quality of seed for which they pay.

To find the genetic similarities in different cotton genotypes was the main objective of this research to resolve the ultimate problems od seed adulterations in cotton seed market. Therefore, the present study has been conducted to estimate the genetic diversity among *Bt* cotton genotypes through SSR markers and to check cotton seed adulteration through genetic purity

Table 1: Names and sources of 46 *Bt* cotton genotypes used in the study

Sr. No.	Name of genotype	Source	Sr. No.	Name of genotype	Source
1	*Bt*-S-15	Farmer field	24	FH-4243	AARI
2	VH-148	CRS, Vehari	25	IUB-222	IUB
3	MNH-456	CRS, Multan	26	*BT*-S-3	Farmer field
4	FH-119	AARI	27	VH-283	CRS, Vehari
5	NS-131	Neelum seed, Multan	28	NIAB-2009	NIAB
6	IR-4	NIBGE	29	VH-289	CRS, Vehari
7	MNH-886	CRS, Multan	30	AS-01	Farmer field
8	IR-3701	NIBGE	31	VH-295	CRS, Vehari
9	*Bt*-122	Farmer field	32	*Bt*-82/8	Farmer field
10	*Bt*-212/10	Farmer field	33	*Bt*-20/289	Farmer field
11	VH-259	CRS, Vehari	34	*Bt*-149	Ali Akbar, Multan
12	FH-118	AARI	35	*Bt*-214/10	Farmer field
13	AA-802	Ali Akbar, Multan	36	*Bt*-2333	Farmer field
14	NS*Bt*-121	Neelum seed Multan	37	*Bt*-23	Neelum seed Multan
15	VH-282	CRS, Multan	38	FH-187	AARI
16	FH-114	Farmer field	39	FH-177	AARI
17	N-820	Farmer field	40	FH-171	AARI
18	IR-3	NIBGE	41	FH-167	AARI
19	IR-901	NIBGE	42	FH-161	AARI
20	MNH-888	CRS, Multan	43	FH-182	AARI
21	AA-703	Ali Akbar, Multan	44	FH-153	AARI
22	FH-113	AARI	45	FH-183	AARI
23	FH-114	AARI	46	FH-158	AARI

estimation of the *Bt* cotton seed o*Bt*ained from different sources in Pakistan.

2. Materials and Methods

2.1. Germplasm collection and DNA extraction:

This research work was conducted in the Plant Genetic Resource (PGR) lab, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. Forty-six elite genotypes of *Bt* cotton collected from different sources in the cotton belt of Pakistan were used in this study (Table 1). Young tender leaves of all the genotypes were collected from the field, washed with d_3H_2O, dried and placed in -80ºC refrigerator in zipper bags till used. DNA was extracted using modified CTAB (Cetyle trimethyl ammonium bromide) method (Azmat et al., 2012).

2.2. Primer Sequences:

Primer pairs were selected From CMD website (http://www.cottonmarker.org/) based on their abundance in cotton genome and polymorphism content. Twenty-two primer pairs were used in this experiment (Table 4).

2.3. SSR Analysis:

PCR reaction (25µl) contained 30ng/µl DNA; 10X buffer; 10Mm dNTPs; 25mM $MgCl_2$; 30ng/µl of each of forward and reverse primers. PCR was performed in thermal cycler using a denaturation step of 4 min at 94ºC followed by 40 cycles of denaturation of 1 min at 94ºC, annealing at 55ºC for 1 min and elongation at 72ºC for 1 min. the program ended with final elongation at 72ºC for 8 min. the amplified products were separated by electrophoresis and polymorphism was detected on 2% agarose gel using ethidium bromide and polyacrylamide gels by silver staining. Gels were observed by UV Transilluminator and photographed using Syne Gene® Gel documentation system.

2.4. Data Analysis:

The PCR products obtained after SSR analysis were scored on the basis of presence (1) and absence (0) of bands for all the genotypes. Ambiguous bands that could not be distinguished clearly were not scored. Genetic divergence and cluster analysis was performed by using MVSP

Table 2: PIC value range and Informativeness of the 22 SSR markers in accordance with PIC values for 46 *Bt* cotton genotypes

Marker system	Ranges of PIC value for *Bt* cotton genotypes		
SSR	Uninformative ≤ 0.30	Moderately informative (0.30-0.59)	Highly Informative ≥ 0.60
	6 (27.3%)	15 (68%)	1 (4.5%)

Table 3: PIC value of primers per loci for 46 *Bt* cotton genotypes

Sr. No.	Primer Name	PIC Value per loci	Sr. No.	Primer Name	PIC Value per loci
1	BNL137	0.00	**12**	BNL1495	0.500
2	BNL285	0.402	**13**	BNL1551	0.673
3	BNL597	0.01	**14**	BNL1606	0.340
4	BNL598	0.347	**15**	BNL1666	0.00
5	BNL686	0.347	**16**	BNL1669	0.144
6	BNL827	0.510	**17**	BNL1672	0.250
7	BNL1122	0.429	**18**	BNL1707	0.538
8	BNL1163	0.347	**19**	BNL2634	0.347
9	BNL1231	0.00	**20**	JESPR290	0.347
10	BNL1317	0.516	**21**	JESPR291	0.347
11	BNL1434	0.420	**22**	JESPR292	0.510

Table 4: Primer names, sequences and number of amplified alleles by 22 SSR Markers in 46 *Bt* cotton genotypes.

Sr. No.	Primer Name	Sequence (5′-3′)	No. of Amplified Alleles	No. of Polymorphic alleles	No. of Monomorphic Alleles
1	BNL137 F BNL137 R	CCCCTTTTACTCTGATTTGGCTT CTTATCGCAATAGTCGCAGTAAT	01	0	01
2	BNL285 F BNL285 R	ACGCTGTTGATAGAGAGAAATACC TCACCGTCCGTTTTAACACA	02	02	0
3	BNL597 F BNL597 R	CCCATCCCTTCATAAACCCT GGGATTGAATATCTCGGCAA	02	0	02
4	BNL598 F BNL598 R	TATCTCCTTCACGATTCCATCAT AAAAGAAAACAGGGTCAAAAGAA	03	02	01
5	BNL686 F BNL686 R	ATTTTTCCCTTGGTGGTCCT ACATGATAGAAATATAAACCAAACACG	03	02	01
6	BNL827 F BNL827 R	AAGCTCCACGTGCTCAAGTT CTCATGTTGTCGGTGGTGTT	02	02	0
7	BNL1122 F BNL1122 R	TCGATAACGGCTATAGTAATCTCTC CAACAAATAAGCAGCCAAGAAA	04	03	01
8	BNL1163 F BNL1163 R	CCAAGCTCTCATCAACACGA TTTTTTCTTTTTCCATTTAAGGG	03	03	0
9	BNL1231 F BNL1231 R	TAATAAAAGGGAAAGGAAAGAGTT TATGGCTCTAGAATATTCCCTCG	01	0	01
10	BNL1317 F BNL1317 R	AAAAATCAGCCAAATTGGGA CGTCAACAATTGTCCCAAGA	04	04	0
11	BNL1434 F BNL1434 R	AAATTCAAGAATCAAAAAACAACA TTATGCCAAAGTATATGGAGTAACG	03	03	0
12	BNL1495 F BNL1495 R	TGAAGATTTGGAGGCAATTG ATAAATGGCATCAGCCCAAA	02	02	0
13	BNL1551 F BNL1551 R	CGCAAGCCACCTGTAAAAC TCGAATTTTCTCTCTCTCTCTCTCT	03	03	0
14	BNL1606 F BNL1606 R	CATGTAGGATGAGAGAGAGAGAGAGA GGGGCTTTACGACATACCTG	03	02	01
15	BNL1666 F BNL1666 R	TGTCAGAAAAGTTTTTCCAAGG AGATCATATTTAAAAGAAAAAGAAAACC	01	0	01
16	BNL1669 F BNL1669 R	AAAGGGATATTGGGTGTGCA TCGAGCATGTGCTCATTGAT	03	01	02
17	BNL1672F BNL1672R	TGGATTTGTCCCTCTGTGTG AACCAACTTTTCCAACACCG	02	02	0
18	BNL1707F BNL1707R	TCCTAGGCTGAGTGAGGGCT AATGACGTCGTTTTATGCCC	04	04	0
19	BNL2634F BNL2634R	AACAACATTGAAAGTCGGGG CCCAGCTGCTTATTGGTTTC	03	02	01
20	JESPR290 F JESPR290R	ACCGGTCAGTCCTCATAATC GCCAAGGTCGTAGTCCAGG	03	02	01
21	JESPR291 F JESPR291R	CATTCCCCACTTTGCTCTTAC CATGTTTCTTTGCCCATC	02	02	0
22	JESPR292 F JESPR292R	GCTTGCAATCTCCTACACC GAATATGTTTCATAGAATGGC	02	02	0

software. Dendrogram were constructed by Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. The Polymorphism Information Content (PIC) was calculated by using the following equation (Botstein et al., 1980):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2 = 1 - \sum_{i=1}^{n} p_i^2 - \left(\sum_{i=1}^{n} p_i^2\right)^2 + \sum_{i=1}^{n} p_i^4 \ ,$$

3. Results:

Forty-six *Bt* cotton genotypes were analyzed by using 22 SSR primer pairs. Each primer-template produced distinct,

easily detectable bands of varying intensities. Unclear bands produced by nonspecific amplification were ignored. Total 56 number of bands were produced, out of which 43 were polymorphic showing 76.78% polymorphism. The number of bands per primer ranged from 1 to 4 with the average of 2.5 bands per primer. The maximum number of bands was produced by the primers BNL-1122, BNL-1317 and BNL-1707 while the minimum number of bands was produced by BNL-137, BNL-1231and BNL-1666 primers (Table 4).

Polymorphism Information content is the property of DNA markers which describes the polymorphism found within the population. Its value depends upon the number of alleles and their frequency. Mostly, the high PIC value of primer shows the presence of variability in the population. The PIC value ranged from 0 to 0.673 with the average value of 0.332 (Table 2). The BNL-1551 primer had the highest PIC value of 0.673 which shows its highest level of polymorphism So, it is concluded that BNL-1551 has more potential to discriminate different genotypes. The lowest PIC value was observed in case of BNL-137, BNL-1231 and BNL-1666 (Table 3)

The informativeness of the SSR primers for 46 *Bt* cotton genotypes was detected based on their PIC values. Only one SSR primer BNL- 1551 was found as highly informative in *Bt* cotton genotypes. Fifteen primers were moderately informative in detecting polymorphism in *Bt* cotton genotypes and remaining six primers did not provide any information about polymorphic variation in *Bt* cotton genotypes (Table 2).

Dissimilarity matrix was generated by MVSP software for 46 *Bt* cotton genotypes. The range of dissimilarity values was from 0.141 to 0. 547 with the average value of 0.392, which depicted the low level of genetic divergence among 46 *Bt* cotton genotypes. Highest dissimilarity coefficient (0.547) was found between IUB-222 and FH-167, which suggests that they may have a different genetic background. The lowest dissimilarity coefficient (0.141) was found between VH-282 and AS-01 *Bt* cotton genotypes which showed that they had genetically close parental lines. Dendrogram was constructed by Unweighted Paired Group of Arithmatic Means Averages (UPGMA) method to find the genetic relatedness between these *Bt* cotton genotypes. All genotypes were divided into four main clusters (Figure 1). Cluster A was further divided into two subclusters. The subcluster A_1 consisted of MNH-888, *Bt*-121, FH-114 and *Bt*-122. The genotypes FH-114 and *Bt*-122 were more closely located which shows higher similarity than others. The subcluster A_2 consisted of IR-4, FH-167, FH-171, FH-118, FH-153, NS-131and FH-119. The genotypes NS-131and FH-119 were the most similar genotypes of group A. It suggests that they may have the same origin. IR-4 was an outlier which means that the IR-4 was the most diverse genotype found in cluster A.

Cluster B was further divided into three subclusters. The subcluster B_1 contained FH-158, FH-4243, *Bt*-20289, AS-01 and VH- 282. The genotypes FH-158 and FH-4243 were similar to each other while the remaining three genotypes were more similar with each other. The genotypes AS-01 and VH- 282 were 85% similar to each other. The close similarity between them being from the same region shows the narrow genetic base of cotton. The subcluster B_2 consisted of AA-802, NIAB-2009 and VH-259. The AS-01 and VH- 282 genotypes were more similar with each other than AA-802.While the subcluster B_3 consisted of FH-114 (Farmer field), *Bt*-S-3, IUB-222 and *Bt*-212/10 genotypes. The IUB-222 and *Bt*-212/10 genotypes were clustered together while FH-114 and *BT*-S-3 remained un-clustered which showed their diverse nature than others.

Cluster C was also divided into three subclusters. VH-295 and IR-3 were clustered together in subcluster C_1. *Bt*-2333, *Bt*-23, *Bt*-214/10 and SB-149 were

present in subcluster C_2. The genotypes *Bt*-23, *Bt*-214/10 and SB-149 were clustered together while *Bt*-2333 remained un-clustered. The genotypes FH-187, N-820, IR-3701 and MNH-886 were present in subcluster C_3. IR-3701 and MNH-886 were clustered together. They had the highest similarity among them than other genotypes present in group C. While FH-187, *Bt*-N-82 genotypes remained un-clustered which showed their diverse nature in contrast to other genotypes present in subcluster C_3.

Cluster D was further divided into four subclusters. In subcluster D_1 FH-177, VH-289 and VH-283 were present. The genotypes VH-289 and VH-283 were clustered together while FH-177 remained un-clustered. The subcluster D_2 consisted of FH-182, *Bt*-82/8, FH-183 and MNH-456 *Bt* cotton genotypes. The genotypes FH-182, *Bt*-82/8 were grouped together while FH-183 and *Bt*-456 genotypes grouped together. The third subcluster D_3 contained the genotypes FH-161 and FH-113 which were clustered together. While the fourth subcluster D_4 consisted of AA-703, IR-901 and VH-148. The *Bt* cotton genotypes IR-901 and VH-148 grouped together, and AA-703 was outlier which showed its distinct behavior than the other genotypes present in cluster D.

between cotton genotypes belonging to different species (Boopathi et al., 2008; Azmat and Khan, 2010; Mostafa et al., 2011; Ullah et al., 2012; Dahab et al., 2013). In the present study, 22 SSR marker loci were used to find the genetic divergence of 46 *Bt* cotton genotypes. Considering 22 primer sets and all the *Bt* genotypes, 43 polymorphic bands were produced showing 76.78% polymorphism which are comparable with previously studied 204 SSR markers that produced 122 polymorphic bands in 40 non-*Bt* cotton varieties (Khan et al., 2009). In another study, 46 polymorphic bands were observed by the 21 SSR primers in 19 varieties of G. *hirsutum* (Azmat and Khan, 2010). The number of bands per primer ranged from 1 to 4 with an average of 2.5 bands per primer. Presence of one to seven alleles per SSR loci have been previously reported (Kantartzi et al., 2009; Azmat and Khan, 2010). The PIC value ranged from 0 to 0.673 with the average value of 0.332. The highest PIC value was found for the primer BNL-1551, which shows that this primer has a potential to discriminate these *Bt* genotypes. The average PIC value was greater than the PIC value (0.29) found within the cultivated cotton species of G. *hirsutum* (Yu et al., 2012). In another study

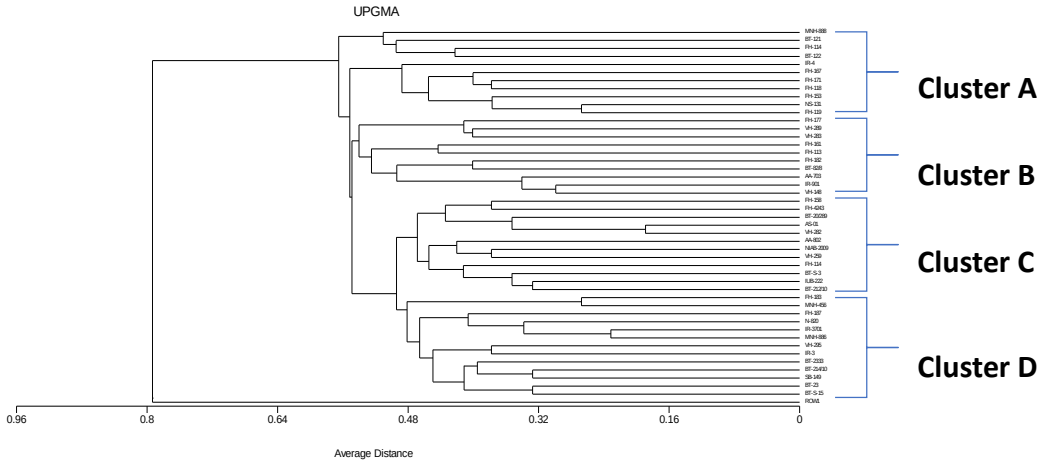

Figure 1: Dendrogram representing the relationship of 46 *Bt* cotton genotypes generated by Unweighted Pair Group Method by using Arithmetic Averages (UPGMA) by using twenty-two simple sequence repeat primers.

4. Discussion

Many studies have been carried out to evaluate the genetic distances among and

the PIC value ranged from 0.18 to 0.62 with an average of 0.40 (Bertini et al., 2006). Average value of polymorphism

information content was 0.62 in an experiment done by Guang and Ming (2006). In contrast to previous results, low range of PIC value in the present study may show the narrow genetic base of selected genotypes. In a study done by Azmat and Khan (2010), the average PIC value of SSR markers was 0.29 in G. *hirsutum* which also exhibited the low genetic base of cultivated cotton. The fluctuation in the polymorphism information content may reflect the genetic nature of the material under study, as the extent of polymorphism differs significantly between and within the species.

Genetic diversity in population of a particular organism is a basic requirement to cope with ecological changes and it is generally attributed by the rate of polymorphism. It is usually measured as the average sequence divergence between any two genotypes for a given loci (Wendel and Brubaker, 1993). In the present study the value of genetic similarities in *Bt* cotton genotypes ranged from 0.453 to 0.859 with an average of 0.607. This high similarity shows the narrow genetic nature of *Bt* genotypes under study. The maximum similarity was found between AS-01and VH-282 genotypes which suggest that they may have originated from the closely related parents. The results of genetic diversity estimates obtained in previous study (Ullah et al., 2012) also showed a very high similarity of 90.2 to 98.2 in the 19 *Bt* cotton varieties. Low genetic variation in cotton is also reported by many previous studies (Lacape et al., 2007; Yasmin et al., 2008; Ullah et al., 2012). In another genetic diversity analysis, the uniformity value ranged from 0.50 to 0.98 with the mean value of 0.83 for 20 genotypes including *Bt* and non *Bt* cotton varieties (Ali et al., 2011). Comparatively low similarity values obtained in the present study in comparison to previous results may be due to the relatively high number of genotypes used in this study, which included 46 *Bt* cotton genotypes collected from farmer fields in Pakistan.

The genotypes FH-114 (Farmer field) and FH-114 (AARI) were grouped in different clusters, which means that they are different from each other. This situation shows that FH-114 (Farmer field) is a different genotype present in the field but with the same name. Thus, there is problem of adulteration of cotton seed in the market which should be resolved to ensure the genetic purity of seed.

The genotypes MNH-888, *Bt*-121 and IR-4 showed distinct behavior than the other *Bt* genotypes, which means that they are diverse in nature. They can be exploited in breeding programmes as potential parents to increase *Bt* cotton diversification. In another study Ali et al., (2011) some *Bt* and non-*Bt* diverse genotypes were found. These genotypes named SARMAST, MNH-786, IR-1524 and FH-1000 were dissimilar from the other genotypes and can be used in future breeding programmes.

The monoculture of some successful varieties has created genetic uniformity in cotton. Modern cotton varieties have been developed by repeated hybridization of closely related genotypes, selection for good quality and yield traits, and recurrent selection within varieties, which have led to further similarity (Van Esbroeck et al., 1998; Bowman, 2000). The prominence of Pakistani developed cotton is even more uniform which can be analyzed by looking into the recent breeding history of Pakistani cotton. Most of the cotton varieties until the early 1990s were developed through crosses of local cultivars with *G. hirsutum* (American cotton) varieties. This produced relatively less diverse cotton varieties. The exotic *Bt* cotton, having insect-resistance gene, had come in Pakistan in 1998 through unknown sources which gained the popularity among cotton farmers. Then local *Bt* varieties were developed by crossing the exotic *Bt* variety with local germplasms (Rahman et al., 2002).

The high similarity among the genotypes showed narrow genetic base of Pakistani developed cotton, apparently because of having at least one common parent. Results

of the present study are in accordance with the previous study done by (Ullah et al., 2012) which indicated a low level of genetic diversity among Pakistani-bred *Bt* cotton. The low level of variation may make them susceptible to potential threats such as CLCuD, which impose the need to introduce genetically diverse parents in cotton breeding programme to enhance genetic variability for sustainable and good quality lint production.

5. Conclusion

It is concluded that the genetic diversity of *Bt* cotton is decreasing due to use of selective parents in breeding programme. Low level of variations would lead the *Bt* cotton toward homogeneity and this high uniformity may cause threat to cotton varieties as high similarity may make them vulnerable to various biotic and abiotic stresses. This can direct permanent damage of the whole cotton crop in the country. This alarming situation requires the urgent need to widen the genetic base of *Bt* cotton. There is a need for the use of diverse genetic material in *Bt* cotton development, or other breeding techniques such as mutation breeding or introgression breeding should be adopted to cause genetic diversity to introduce wild genes in cultivated cotton germplasm. Moreover, strict and wise measurements are required by the government to address seed adulteration in marketing system, where the seed of similar genotypes are sold under different name labels. Genetic purity of the seed should be ensured with the help of molecular breeding techniques to preserve the breeder's rights.

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