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ASSESSMENT OF MORPHOLOGICAL AND MOLECULAR MARKER BASED GENETIC DIVERSITY AMONG ADVANCED UPLAND COTTON GENOTYPES

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Cotton is an essential fiber and oilseed crop cultivated in semi-dry to arid atmosphere rage in various parts of the world. For a successful breeding program, it is of extreme significance for the plant breeders to have knowledge of genetic variability present in the breeding material. Therefore, present investigations were carried out to make sense of genetic relatedness and differences on the basis of morphological traits (tap root length, lateral root number and root volume) and molecular markers (SSR) among 30 upland Bt cotton genotypes developed by public and private sector. Results of morphological data indicated that maximum Tap root length, lateral root numbers and root volume were recorded for genotypes BH-178, BH-180 and CIM-602 respectively. So, these genotypes may be used in breeding program to improve respective trait. Meanwhile for molecular study Out of 40 markers, 20 were found polymorphic among 30 genotypes. Total number of alleles opened up by these markers were 116 while 90 were found polymorphic demonstrating 22% genetic diversity with 4.50 alleles for each locus on an average. Dendrogram demonstrated a most extreme range of closeness from 78 to 98%. Least likeness (78%) was seen among the lines FH-142 and TARZAN-1, though, most extreme (98 %) was seen between the lines FH-113 and FH-114. It is direly expected to incorporate exotic cotton germplasm to expand magnitude of genetic variability which may bring improvement in seed cotton yield and other economic traits in cotton crop.

Keywords: Upland cotton, Bt cotton, germplasm, genetic variability, molecular markers

INTRODUCTION

Cotton is a vital fiber and oilseed crop in Pakistan. American cotton (*Gossypium hirsutum* L.) is an imperative common fiber that spreads around 90% of the zone under cotton development around the world (Khadi *et al*., 2009). Genetic variability is an imperative criterion for the upgradation of existing plant germplasm and development of new cultivars through the determination of different parental lines for new crosses. The narrow genetic base of tetraploid cotton is one of the central point influencing yield and nature of the harvest (Van-Esbroeck and Bowman, 1998). The problem can be overcome by using modern tools for exploiting genetic diversity in cotton to reveal the molecular basis of the traits of agronomic importance.

Molecular markers are believed to be versatile tool to study genetic diversity among different plant species and identify potential genotypes for a successful breeding program (Saghai-Maroof *et al*., 1984; Tatineni *et al*., 1996; Ercan *et al*., 2018). A class of genetic markers known as Simple Sequence Repeat (SSR) is proved to be highly polymorphic (Khandagale *et al.,* 2007; Dongre *et al.,* 2011) and is successfully used in cotton genome mapping and marker assisted selection (Han *et al.,* 2006). Previously, morphological and biochemical markers were used to estimate genetic diversity but these were influenced by environmental factors. Molecular markers have provided best systems for the analysis of genetic diversity (Zhang *et al*., 2011). In this system, initially, PCR-based markers were used to analyze genetic diversity i.e. Random Amplified Polymorphic DNA (RAPD) (Lu and Myers, 2002) and Amplified Fragment Length Polymorphism (AFLP). But with the passage of time, Simple Sequence Repeats (SSR) (Zhu *et al*., 2003; Kantartzi *et al*., 2009), inter Simple Sequence Repeats (ISSR) (Reddy *et al*., 2002; Noor Mohammadi *et al*., 2014), and Single Nucleotide Polymorphism (SNP) (Van Deynze *et al*., 2009) have made this system more convenient and reliable. Molecular markers are believed to be versatile

tool to study genetic diversity among different plant species and identify potential genotypes for a successful breeding program (Saghai-Maroof *et al*., 1984; Tatineni *et al*., 1996). Simple sequence repeat (SSRs are widely used to assess variability among parental lines due to polymorphic and multiallelic nature. Most recently, microsatellite or simple sequence repeat (SSR are being used to find genetic diversity (Blenda *et al*., 2006; Ahmad *et al*., 2007; Saeed *et al*., 2011; Tyagi *et al*., 2014; Zhao *et al*., 2014) because of their they can be reproducible, transferable and co-dominant nature and have superiority over other markers (Ghaffari *et al*., 2014). Modern plant genomic approaches have enabled plant scientists to study population undercurrents, specific gene surveys, and selection of best genotypes having favorable traits prior to domestication (Glaszmann *et al*., 2010).

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MATERIALS AND METHODS

Thirty Bt cotton genotypes of public and private sector of Pakistan were evaluated at morphological and molecular level for genetic variability. A list of genotypes and their origin information is provided in Table 1. The study was conducted in a greenhouse at the experimental area of the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. Experiment was conducted in earthen pots having, perforated at the base, filled with 5.0 kg of soil (sand: silt: clay in the ratio of 1:1:1) and well decomposed farm yard manure mixed. Pots were watered to field capacity before planting. NPK fertilizer (23:21:0 + 4S) was thoroughly mixed in water and added to each pot prior to planting at rates equivalent to 34 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5, and 22 kg ha^{-1}. Seedlings were thinned to one plant per pot, three weeks after planting. Plants were allowed to grow under optimum water regime from sowing to harvesting. After 60 days of planting data were recorded for morphological traits (Tap root length, lateral root number and root volume) and fresh leaf samples were picked for molecular studies with SSR markers

***DNA extraction*:** Fresh leaf samples of all the parental genotypes were taken from the field for DNA extraction. Leaf samples of selected plants were picked, packed in plastic bags and instantly moved to freezer at -80°C. Doyle and Doyle (1990) standard CTAB method was used with some modifications to extract DNA. About 5 gram5-gram leaf sample of each genotype was ground with preheated 2 × CTAB extraction buffer (65°C) and paste was transferred to 50 ml polypropylene tube and incubated in water bath at 65°C for 50 minutes. In the next step, an equal volume of chloroform: isoamyl alcohol (24:1) was added to paste and gently mixed by inverting the tube. The emulsion was centrifuged for 10 min at 5000 rpm and supernatant was transferred to a new tube. Chilled propanol of 0.6 volumes was added to the new tube and centrifuged for 10 minutes at

Table 1. 30 upland Bt cotton genotypes and their origin.

Sr. No	Genotype	Origin	Sr. No	Genotype	Origin
1	MNH-886	AARI, Faisalabad	16	TARZAN-1	4B, Pvt. Ltd
2	AGC-777	AARI, Faisalabad	17	IUB-222	IUB, Bahawalpur
3	BH-178	Bahawalpur	18	KZ-181	Kanzoo Pvt. Ltd
4	FH-118	AARI, Faisalabad	19	LEADER-1	Suncrop Pvt. Ltd
5	FH-113	CRS, Multan	20	FH-114	Allah Din Pvt. Ltd
6	CIM-598	CCRI, Multan	21	MM-58	Vehari
7	CIM-600	CCRI, Multan	22	A-555	Allah Din Pvt. Ltd
8	CIM-606	CCRI, Multan	23	NIAB-824	NIAB, Fsd
9	BH-184	Bahawalpur	24	NIBGE-901	NIBGE, Fsd
10	FH-142	AARI, Faisalabad	25	NIBGE-3	NIBGE, Fsd
11	FH-LALAZAR	AARI, Faisalabad	26	MNH-988	CRS, Multan
12	CIM-602	CCRI, Multan	27	IUB-13	IUB, Bahawalpur
13	VH-259	ARI, Vehari	28	BH-180	Bahawalpur
14	CEMB-33	CEMB, Lahore	29	MNH-456	CRS, Multan
15	BS-52 B1	Bahawalpur	30	VH-301	ARI, Vehari

5000 rpm. After centrifugation the supernatant was discarded and pellet was washed with 70% alcohol. The pellet was dried before dissolving in double distilled water for RNase treatment. To digest RNA, 5 µl RNase (10 mg/ml) was added to the dissolved pellet and quality of DNA samples was checked by gelelectrophoresis. The DNA samples giving smear in the gel were rejected and only good quality DNA was selected for polymerase chain reaction.

***SSR analysis*:** For SSR examination, a reaction volume of 20 µL was made containing 2.0 µL 10X polymerase chain reaction support (50 mM Tris, pH 8.3, 500 mM KCl); 1.5 mM MgCl2; 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Fermentas, USA); 0.6 µM each of forward and reverse primers (GeneLink, USA); Taq DNA polymerase (Fermentas), and 50 ng genomic DNA as a layout. Enhancement was performed in a Mastercycler Gradient (Eppendorf, Germany) with the accompanying temperature cycles: first denaturation venture of 94°C for 5 min took after by 35 cycles of 30 s at 94°C, 30 s at around 55°C (changed with the dissolving temperature of the markers), and 1 min at 72°C. A last augmentation was performed at 72°C for 5 min. Intensified items were settled on 6% polyacrylamide gel (19:1, acryl:bis) on an OmniPAGE Maxi Vertical Electrophoresis framework (Biocom Direct, UK) and envisioned with silver recoloring. To examine the genetic connections among the 30 Bt cotton genotypes measured, we considered each band a solitary locus/allele. Hereditary similitude frameworks were produced in light of Nei's coefficients (Nei, 1972). NT Syspc 2.0 was utilized for dendrogram development with the unweighted-pair group method of arithmetic means (UPGMA).

RESULTS

***Morphological traits based results*:** Mean values for different morphological traits of cotton genotypes were calculated for comparison (Table 2). Maximum tap root length was recorded for CEMB-33 (45.41) followed by BH-178 (45.40), FH-142 (44.91), CIM-600 (44.53), FH-113 (44.17) and CIM-606 (43.53) while lowest length was found for kz-181 (34.33).

Table 2. Mean values of 30 upland cotton genotypes for morphological traits.

Sr.	Genotypes	Tap root length	SE	Lateral root number	SE	Root volume (mm^3)	SE
1	MNH-886	41.92	0.421	30.33	0.700	3.53	0.322
2	AGC-777	42.63	0.418	29.43	0.724	4.10	0.297
3	BH-178	45.40	0.411	25.47	0.786	4.43	0.291
4	FH-118	42.10	0.415	25.03	0.806	3.37	0.336
5	FH-113	44.17	0.411	26.27	0.798	4.27	0.299
6	CIM-598	42.32	0.417	23.73	0.856	4.27	0.303
7	CIM-600	44.53	0.412	31.63	0.747	4.47	0.301
8	CIM-606	43.53	0.408	29.21	0.774	5.07	0.285
9	BH-184	42.50	0.411	31.47	0.757	4.43	0.289
10	FH-142	44.91	0.404	29.27	0.781	4.57	0.285
11	FH-LALAZAR	35.50	0.433	26.83	0.828	4.12	0.297
12	CIM-602	41.67	0.372	25.37	0.873	5.10	0.272
13	VH-259	37.23	0.402	23.27	0.935	4.27	0.266
14	CEMB-33	45.40	0.356	25.70	0.901	4.20	0.269
15	BS-52	42.67	0.327	27.03	0.904	3.93	0.280
16	TARZAN-1	38.46	0.340	25.10	0.969	3.27	0.315
17	IUB-222	42.10	0.329	23.37	1.035	3.32	0.317
18	KZ-181	34.33	0.367	31.81	0.903	3.07	0.337
19	LEADER-1	43.09	0.217	34.07	0.876	4.07	0.288
20	FH-114	41.30	0.195	22.10	1.028	4.77	0.275
21	MM-58	39.27	0.203	20.47	1.079	2.97	0.309
22	A-555	37.37	0.212	22.17	0.994	3.27	0.280
23	NIAB-824	41.17	0.128	25.70	0.907	3.53	0.271
24	NIBGE-901	41.11	0.133	22.03	1.032	4.01	0.267
25	NIBGE-3	39.87	0.142	25.32	0.900	3.22	0.319
26	MNH-988	40.23	0.146	23.37	0.963	3.43	0.304
27	IUB-13	39.98	0.160	24.87	0.801	3.67	0.303
28	BH-180	42.27	0.165	35.77	0.447	4.27	0.310
29	MNH-456	41.21	0.105	29.42	0.258	3.10	0.434
30	VH-301	40.11	0.015	31.21	0.122	4.53	0.083

Findings for lateral root number showed that maximum lateral root number was recorded for BH-180 (35.77) followed by LEADER-1 (34.07), KZ-181 (31.81), CIM-600 (31.63), BH-184 (31.47) and VH-301 (31.21) while lowest length was found for MM-58 (20.47). Maximum root volume was recorded for CIM-602 (5.10) followed by CIM-606 (5.07), AGC-777 (4.77), FH-142 (4.57), VH-301 (4.53) and CIM-600 (4.47) while lowest length was found for MM-58 (2.97). Eigen value for tap root length was 1.659, 0.838 for lateral root number and 0.503 for root volume (Fig. 1).

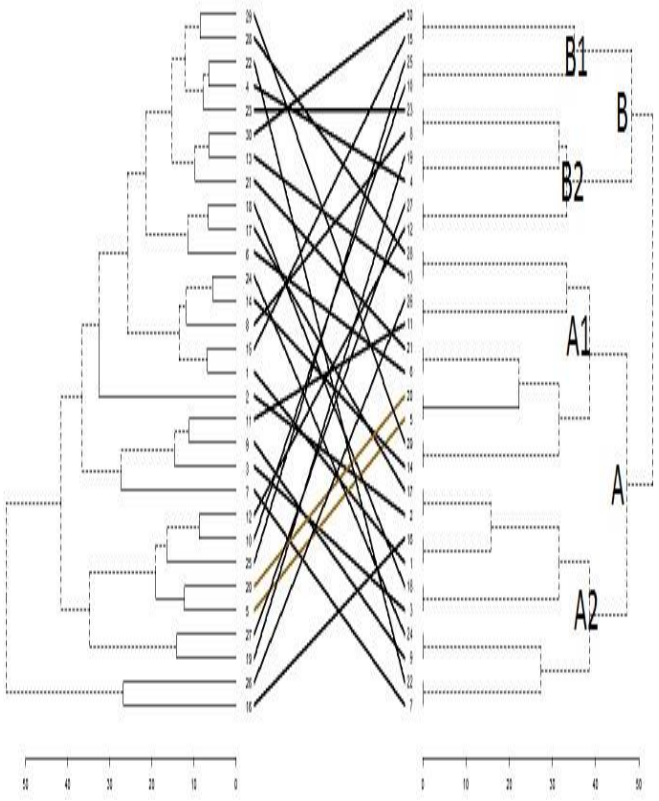

Figure 1. Comparative dendrogram of 30 upland cotton genotypes for SSRs (Right) and morphological data (Left).

Cumulative percentage of 55.31% and 83.23% for tap root length was recorded for lateral root number and 100 for root volume (Figure 1). According to morphological data bi-plot analysis genotypes IUB-222, BH-180, FH-142, BS-52 and NIBGE-901 were found to be present in coordinate-1; genotypes IUB-13, KZ-181, MNH-456, CIM-606, MNH-886 and CIM-598 were in coordinate-2 and genotypes MM-58, FH-114, AGC-777, CIM-602, A-555, MNH-988, TARZAN-1, NIAB-824 and NIBGE-3 were in coordinate-3 while genotypes CEMB-33, CIM-600, BH-184, FH-118, FH-LALAZAR, BH-178, FH-113, LEADER-1 and VH-301 were present in coordinate-4 (Fig. 2). Results of comparative dendrogram showed that varieties FH-113 and FH-114 had similar positions in both molecular and morphological dendrograms showing high similarity (Fig. 3).

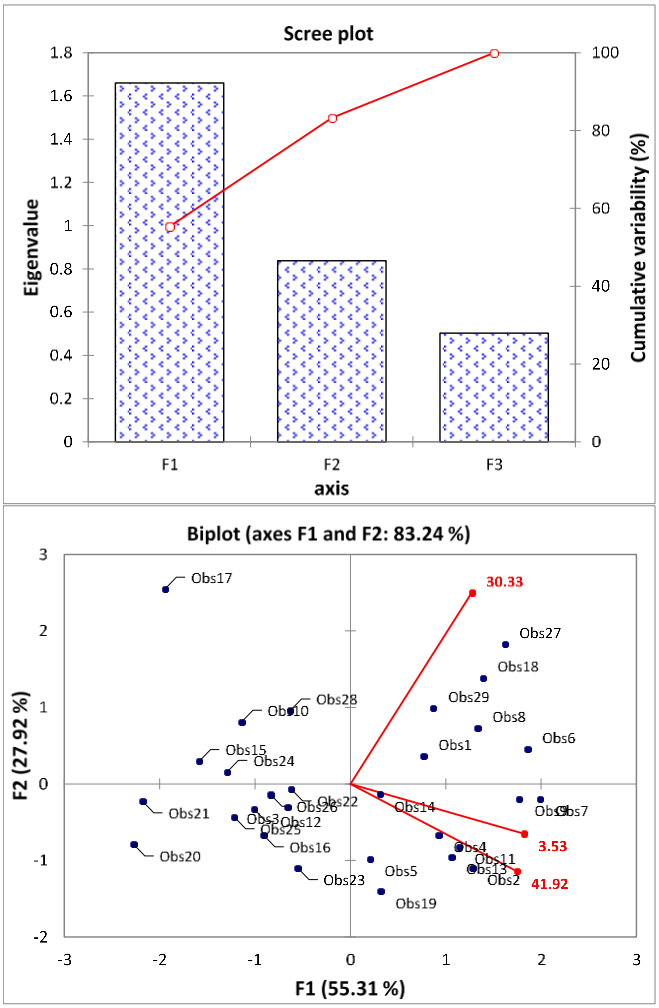

Figure 2. Scree plot and Bi-plot of 30 upland cotton genotypes.

***Molecular marker based results*:** A total of 40 primers were tested for the estimation of genetic diversity among 30 parental genotypes, out of which 20 were found polymorphic. Total number of alleles amplified by these primers were 116 while 90 were found polymorphic showing 22% genetic diversity with 4.5 alleles per locus on an average (Table 3). Dendrogram showed a maximum range of similarity i.e. from 78 to 98% and on average of 87%. Minimum similarity (78%) was observed among the lines FH-142 and TARZAN-1, whereas, maximum (98%) was observed between the lines FH-113 and FH-114 (Fig. 3). On the basis of similarity percentage, the dendrogram was divided into two major groups, i.e. group A and group B. Both group A and B were further subdivided into A1, A2 and B1, B2. Group B comprised of 6 genotypes namely FH-114, FH-113, BH-178,

Table 3. Similarity matrix of 30 upland cotton genotypes.

	FH-113	FH-114	BH-178	FH-118	MNH-886	CIM-598	CIM-602	CIM-606	BH-184	FH-142	FH-L	CIM-600	VH-259	Tar-1	BS-52	CEBB-33	IUB-222	KZ-181	A-555	AGC-777	MM-58	L-1	NIAB-824	IR-901	IR-3	MNH-988	IUB-13	BH-180	MNH-456	VH-301
FH-113	***	0.98	0.84	0.86	0.89	0.79	0.88	0.84	0.87	0.86	0.83	0.79	0.86	0.92	0.93	0.86	0.91	0.88	0.88	0.82	0.91	0.96	0.88	0.90	0.91	0.83	0.88	0.79	0.91	0.82
FH-114		***	0.83	0.84	0.94	0.93	0.86	0.89	0.94	0.84	0.87	0.88	0.95	0.84	0.80	0.83	0.92	0.91	0.83	0.9	0.89	0.88	0.83	0.79	0.82	0.91	0.85	0.80	0.88	0.90
BH-178			***	0.83	0.84	0.85	0.85	0.91	0.92	0.83	0.89	0.92	0.83	0.79	0.94	0.90	0.92	0.86	0.88	0.87	0.98	0.87	0.83	0.89	0.91	0.88	0.81	0.81	0.88	0.90
FH-118				***	0.88	0.87	0.85	0.91	0.88	0.93	0.83	0.89	0.90	0.84	0.80	0.88	0.91	0.91	0.84	0.88	0.84	0.92	0.94	0.89	0.88	0.84	0.85	0.96	0.88	0.94
MNH-886					***	0.83	0.89	0.86	0.87	0.91	0.94	0.87	0.82	0.84	0.83	0.90	0.88	0.90	0.86	0.93	0.88	0.83	0.93	0.80	0.92	0.91	0.87	0.82	0.88	0.88
CIM-598						***	0.81	0.87	0.94	0.86	0.81	0.84	0.81	0.93	0.93	0.82	0.80	0.85	0.91	0.88	0.88	0.88	0.90	0.81	0.93	0.85	0.90	0.88	0.80	0.88
CIM-602							***	0.94	0.94	0.85	0.93	0.85	0.94	0.84	0.88	0.90	0.85	0.88	0.88	0.87	0.91	0.85	0.88	0.90	0.87	0.93	0.94	0.90	0.90	0.86
CIM-606								***	0.88	0.89	0.87	0.93	0.87	0.95	0.89	0.88	0.91	0.84	0.80	0.86	0.93	0.82	0.79	0.89	0.92	0.85	0.88	0.89	0.85	0.88
BH-184									***	0.95	0.96	0.85	0.94	0.93	0.91	0.85	0.93	0.95	0.94	0.87	0.82	0.89	0.95	0.88	0.87	0.80	0.82	0.80	0.81	0.92
FH-142										***	0.88	0.83	0.93	0.78	0.83	0.94	0.89	0.88	0.87	0.90	0.88	0.88	0.79	0.94	0.94	0.90	0.86	0.82	0.93	0.80
FH-L											***	0.88	0.89	0.89	0.87	0.93	0.84	0.84	0.88	0.91	0.97	0.90	0.88	0.83	0.83	0.84	0.82	0.87	0.81	0.81
CIM-600												***	0.88	0.93	0.88	0.89	0.88	0.82	0.91	0.88	0.83	0.89	0.82	0.88	0.81	0.94	0.93	0.90	0.88	0.84
VH-259													***	0.88	0.82	0.88	0.85	0.89	0.82	0.94	0.84	0.88	0.92	0.87	0.94	0.81	0.89	0.87	0.84	0.80
TAR-1														***	0.93	0.86	0.90	0.86	0.88	0.89	0.91	0.88	0.82	0.89	0.83	0.93	0.88	0.95	0.94	0.93
BS-52															***	0.93	0.89	0.91	0.93	0.88	0.92	0.90	0.89	0.84	0.85	0.88	0.81	0.84	0.89	0.88
CEMB-33																***	0.90	0.93	0.88	0.94	0.89	0.89	0.85	0.81	0.82	0.90	0.91	0.88	0.80	0.88
IUB-222																	***	0.87	0.82	0.91	0.81	0.93	0.95	0.90	0.95	0.87	0.90	0.83	0.81	0.79
KZ-181																		***	0.93	0.88	0.86	0.85	0.89	0.81	0.82	0.89	0.88	0.93	0.84	0.90
A-555																			***	0.87	0.80	0.86	0.87	0.82	0.84	0.88	0.81	0.90	0.84	0.92
AGC-777																				***	0.89	0.88	0.88	0.93	0.94	0.93	0.83	0.85	0.82	0.90
MM-58																					***	0.95	0.87	0.82	0.91	0.84	0.93	0.86	0.88	0.89
L-1																						***	0.90	0.93	0.83	0.94	0.90	0.93	0.90	0.88
NIAB-824																							***	0.89	0.94	0.82	0.90	0.91	0.91	0.93
IR-901																								***	0.95	0.89	0.88	0.82	0.93	0.92
IR-3																									***	0.88	0.82	0.80	0.83	0.88
MNH-988																										***	0.88	0.79	0.89	0.83
IUB-13																											***	0.88	0.88	0.82
BH-180																												***	0.93	0.88
MNH-456																													***	0.83
VH-301																														***

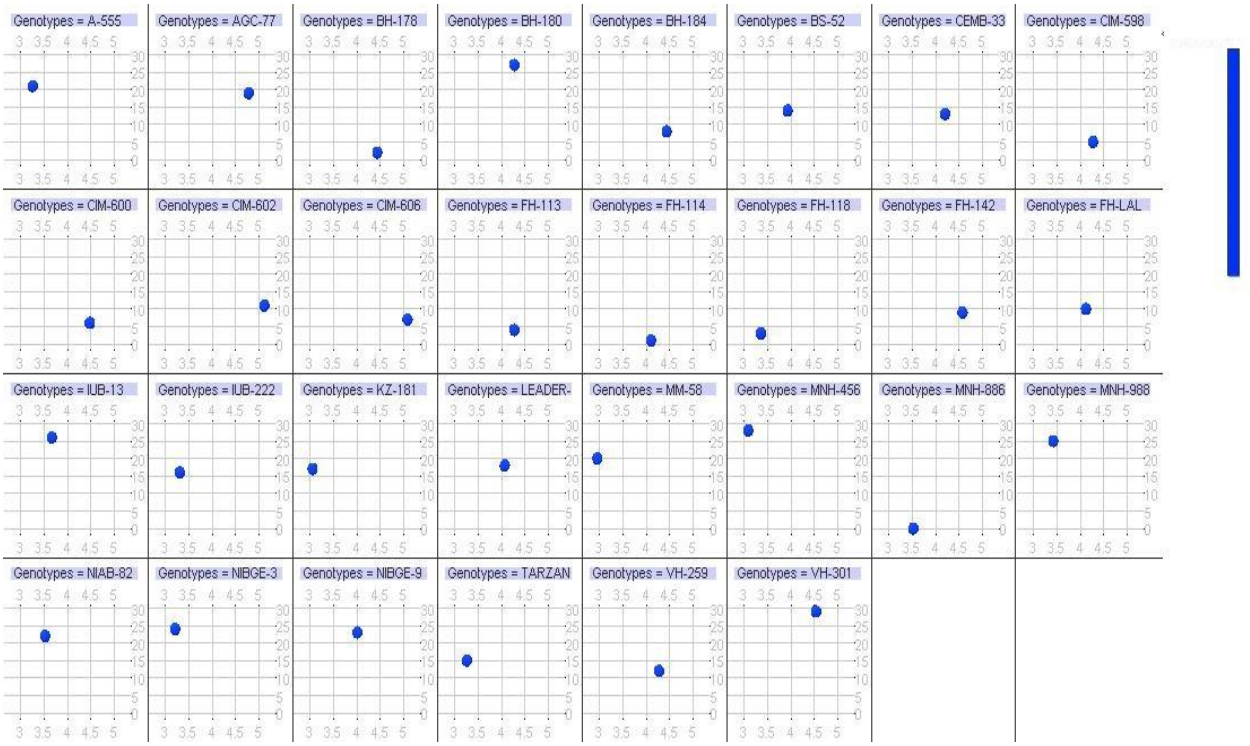

Figure 3. Trellis diagram of 30 cotton genotypes.

CEMB-33, IUB-222 and KZ-181. FH-113 and FH-114 were tolerant lines with 98 % similarity to each other.

Both the lines made a cluster with each other and showed minimum genetic distance. Both genotypes were taken from AARI, Faisalabad. In sub-group A1, fourteen genotypes were present namely BS-52, VH-301, MNH-886, AGC-777, CIM-598, MM-58, LALAZAR, TARZAN-1, BH-184, LEADER-1, VH-259, IUB-13, BH-180 and MNH-988. In sub-group A2, there were ten genotypes i.e. FH-118, A-555, CIM-606, NIAB-824, CIM-600, CIM-602, FH-142, MNH-456, IR-3 and IR-901 (Fig. 3).

Table 4. Scorable DNA bands amplified by polymorphic SSRs through PCR.

Primers	No. of bands	Polymorphic	Polymorphism (%)
NAU862	5	4	80.00
NAU873	7	3	42.85
NAU889	7	4	57.14
NAU915	6	3	50.00
NAU1045	7	4	57.14
NAU967	9	5	55.56
NAU980	3	3	100.00
NAU998	5	4	80.00
NAU1014	6	3	50.00
NAU1023	4	3	75.00
NAU1025	5	4	80.00
NAU1028	7	6	85.71
NAU948	5	3	60.00
NAU943	5	2	40.00
NAU1034	7	2	28.57
NAU1048	6	4	66.67
BNL530	5	4	80.00
BNL 2709	6	4	66.67
BNL 3590	8	4	50.00
BNL 4029	3	2	66.67
Total	116	90	1562.61
Average	5.8	4.50	78.12

DISCUSSION

It has been accounted for that upland cotton developed on the planet is from four varietal classes to be specific Acala, Stoneville, Coker and Deltapine. Out of four, Coker, Deltapine and Stonville have a typical progenitor in the Bohemian range which goes back to 1860 (Niles, 1980). The narrow genetic base of tetraploid cotton is one of the main considerations influencing yield and nature of the harvest (Van-Esbroeck and Bowman, 1998). The problem can be overcome by inputting modern tools for exploiting genetic diversity in cotton to reveal the molecular basis of the traits of agronomic importance. The presence of variability among genotypes for different traits has been reported to be prerequisuit (Basal *et al*., 2006; Iqbal, 2011; Bibi *et al*., 2012). Morphological traits like tape root length, lateral root numbers and root volume have been shown to be increased in varieties that encounter any stress due to stress memory and genetic variability (Pace *et al*., 1999; Chaturvedi *et al*., 2012). Maximum lateral root numbers and root volume were recorded for genotypes BH-180 and CIM-602 respectively in the present investigation. Increased tap root length in response to stress may permit cotton plants to survive by accessing water from deeper layers in the soil profile during periods of limited water supply. In our study, maximum Tap root length was recorded for genotypes BH-178. Although morphological traits have a significant role in assessing genotypic variability but molecular markers provide a precise and accurate idea about the presence of genetic variations in the population. In the present investigations both morphological and molecular techniques had been used to estimate genetic variability. A class of genetic markers (SSR) is proved to be highly polymorphic (Khandagale *et al.*, 2007; Dongre *et al.*, 2011) and is successfully used in cotton genome mapping and marker assisted selection (Han *et al.*, 2006).

Molecular markers are believed to be versatile tool to study genetic diversity among different plant species and identify potential genotypes for a successful breeding program. Molecular markers have a big advantage over morphological, physiological and biochemical markers because these are not affected by environment (Saghai-Maroof *et al*., 1984; Tatineni *et al*., 1996). SSR are widely used to assess variability among parental lines due to polymorphic and multiallelic nature. Cluster between thirty genotypes was constructed on the basis of similarity matrix, the results showed two main groups, i.e. group A and group B. Both the main groups were further subdivided into sub-groups. Khan *et al*. (2009) used SSR markers and grouped forty genotypes into three clusters with an average similarity of 36 to 89%. Ullah *et al*. (2012) utilized 19 cultivars Bt cotton cultivars to determine genetic divergence and found that genotypes were 94% homogeneous indicating narrow genetic base. The findings revealed that the relatedness among cultivars of public sector bred cotton genotypes was much higher than private sector developed lines. So, this similarity could be due to monoculture of some very successful breeding lines adopted well under the present climatic conditions with respect to yield and quality (Van Esbroeck *et al*., 1998). Higher uniformity in recent cultivars is also the result of crossing between closely related parents and repeated selection among better performing genotypes for yield and lint traits.

***Conclusion*:** Genetic similarity of public sector developed Bt cotton genotypes/cultivars was more than private sector genotypes/cultivar. The present standing of developing varieties in Pakistan is more uniform due to selection and breeding of existing germplasm. So, it is urgently needed to create variability through breeding local cultivars with exotic lines to avoid epidemic yield and quality losses. Although cotton has low polymorphism compared to other species but still it has potential for yield and quality breeding.

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