



Genetic Diversity Analysis in Genetically Modified Cotton (*Gossypium hirsutum* L.) Genotypes

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**Abstract:** The present research was aimed to analysis genetic diversity in a set of 26 genetically modified cotton genotypes. The results exhibited that there was significant differences ( $P \leq 0.01$ ) among the genotypes for all the studied traits, showing the substantial genetic variability among the genotypes for further evaluation. With respects to mean performance, variety BH-180 and IR-3701 displayed superior performance in terms of bolls plant<sup>-1</sup>, seed cotton yield plant<sup>-1</sup>, micronaire value, GOT%, staple length and lint index, offering that these genotypes can further be exploited for various breeding programs to improve upland cotton genotypes. High heritability values ( $h^2$  b.s.) were shown by most of the studied traits, indicating that the variation found was primarily under genetic control and was less affected by environmental factors, referring the influence of additive gene action for these traits. Thus, the progress of such traits can be done via direct phenotypic selection. In cluster analysis, all genotypes divided into seven small clusters, cluster one consisted of nine genotypes which were characterized as high yielding genotypes, suggesting that these genotypes could be utilized for future breeding programs in order to get better yielding varieties. First three principal components accounted 99.00% variability, which is considered very high and could be used in upcoming breeding programs of cotton.

**Keywords:** Genetic diversity, heritability analysis, Bt-cotton, seed cotton yield, fiber traits

1. **INTRODUCTION**

Generally in world and particularly in Pakistan, cotton (*Gossypium hirsutum* L.) engages a central position as fiber crop for the masses (Imran *et al.*, 2012). To meet fiber necessities of the world's growing population, a significant improvement in cotton production has been universally realized (Farooq *et al.*, 2013). Therefore, cotton breeders should have more focused for evolving cotton genotypes showing better tolerance against various diseases and pests, greater fiber quality and early maturing related traits. Through the applications of biotechnology, cotton breeders successfully extracted a gene known as Cry1AC from *Bacillus thuringiensis* (Bt) and subsequently introduced into upland cotton with the help of genetic engineering. Cotton genotypes containing Bt gene is generally called Bt-cotton and is predicted to decrease input cost in the shape of less usage of pesticides since this genetically modified organism shows great resistance against bollworm complex (Chandrashekhar, *et al.*, 2015).

Introduction, characterization and recognition of promising breeding materials, is the key step in a crop improvement program. The highly productive in yield with superior adaptation and agronomically desirable characteristics could directly be exploited for commercial cultivation. However, an efficient and substantial hybridization program would be a feasible approach for such a hybridization program to be

(Baloch *et al.*, 2014a). To maintain the high productivity level of any crop, genetic variability offered in nature or created in the course of crop breeding is of immense significance. Genetic similarity within a crop species is readily harbored through the use of same gene or gene complexes during hybridization programs. When genetic similarity happens to the cause of genetic vulnerability, the genetic variability is the only assurance against it. To overcome the threat of this uniformity, it is very necessary that genetic diversity, available in both the cultivated and its wild progenitors, is thoroughly utilized to create new genetic complexes for higher yield and tolerance to various biotic and abiotic stresses (Sanghera *et al.*, 2014). Keeping in view the above mentioned facts, the current study was aimed to assess genetic variability for yield and fiber traits in Bt-cotton genotypes using various statistical approaches so that the promising genotypes could be identified for development of improved cotton varieties.

2. **MATERIALS AND METHODS**

**Plant materials and recorded traits.** The present research was carried out at Botanical Garden, Sindh Agriculture University, Tandojam, Pakistan. The mean performance and genetic diversity was evaluated for some yield and fiber traits in genetically modified cotton genotypes (*Gossypium hirsutum* L.), known as Bt-cotton. The genetic materials consisted of 26 Bt-cotton genotypes, which were sown in

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Randomized Complete Block Design with three replications. The space between plant to plant and row to row was kept at 30 and 75 cm, respectively. All recommended agronomic practices were applied at appropriate time. The plant traits were recorded including bolls plant<sup>-1</sup>, seed cotton yield plant<sup>-1</sup>, micronaire value, ginning outturn percentage, lint index and staple length.

**Data analysis.** Statistix v. 10 software was used to derive analysis of variance, mean comparisons and principal component analysis. Genetic distance was determined by using Popgene software, whereas cluster analysis was conducted with the help of NTSys-pc ver. 1.2 software. Heritability analysis was calculated as proposed by Allard (1960). For cluster analysis, the phenotypic data was converted into 0 and 1 matrix as suggested by Shakhtrah *et al.* (2010). The Bt-cotton genotypes grouped into three groups based on their mean and standard deviations (Shakhtrah *et al.*, 2010). The three groups were:

1. Mean -1 standard deviation.
2. Mean -1 to +1 standard deviation.
3. Mean +1 standard deviation

### 3. RESULTS AND DISCUSSION

**Analysis of variance:** The analysis of variance (Table-1) exhibited that all the tested genotypes performed significantly different ( $P \leq 0.05$ ) for bolls plant<sup>-1</sup>, seed cotton yield plant<sup>-1</sup>, micronaire value, GOT %, lint index and staple length, suggesting that studied

materials possess useful genetic resources for variety of traits thus can extensively be used for upcoming breeding programs. Similar kinds of results have also been reported by other workers including Baloch *et al.* (2014b) and Muhammad *et al.* (2014).

**Heritability analysis:** Estimation of heritability is very essential as it helps in deciding the characters that are being considered while operating the selection. High heritability estimates ( $h^2$  b.s.) were observed for bolls plant<sup>-1</sup> ( $h^2=87.90\%$ ), seed cotton yield plant<sup>-1</sup> ( $h^2=80.90\%$ ), GOT% ( $h^2=71.92\%$ ), staple length ( $h^2=69.72\%$ ) and lint index ( $h^2=66.53\%$ ), whereas low heritability estimates was disclosed by the trait boll weight ( $h^2=40.87\%$ ) (Table-2). Larger heritability values for these traits indicated that the variation observed was principally under genetic control and was less effected by environment cues, referring the influence of additive gene action for these characters. Consequently, the improvement of these traits can be made through direct phenotypic selection. These results are in accordance with recent reports of Hajazi *et al.* (2014), Ahsan *et al.* (2015), Rajamani *et al.* (2015) and Zeng and Pettigrew (2015).

**Cluster analysis:** Cluster analysis is one of the most important statistical analyses which help to do grouping of genotypes based on genetic variability which is existed for various traits. The un-weighted pair group method using arithmetic average (UPGMA) classified the 26 upland cotton into 7 small clusters (Fig. 1).

Table 1. Mean squares from analysis of variance for yield and fiber traits in Bt-cotton genotypes (*Gossypium hirsutum* L).

Source of variation	D.F.	Bolls plant <sup>-1</sup>	Seed cotton yield plant <sup>-1</sup>	Micronaire value	GOT %	Lint index	Staple length
Replications	2	54.0782	234.811	0.02551	15.4594	0.33019	6.29167
Genotypes	25	62.7150**	178.000**	0.03785**	14.6723**	0.35826**	6.01295**
Error	50	2.755	13.091	0.012	1.695	0.051	0.768

Note: \*\* = Significant at 0.01 level of probability

Table 2. Heritability analysis for various traits in Bt-cotton genotypes.

Characters	Genetic variance ( $\delta^2 g$ )	Phenotype variance ( $\delta^2 p$ )	Heritability % (Broad sense)
Bolls plant <sup>-1</sup>	19.80	22.73	87.90
Seed cotton yield plant <sup>-1</sup>	54.97	68.06	80.76
Micronaire value	0.0085	0.0208	40.87
GOT %	4.33	6.02	71.92
Lint index	0.10	0.15	66.53
Staple length	1.74	2.50	69.72

This indicates the presence of wide genetic diversity among the tested genotypes. The first cluster consisted of nine cotton genotypes including BH-180, KZ-389, SB-149, IR-3701, AA919, GH-142, SITARA-12, MM-158, and CEMB-44. This cluster can be characterized by having more number of bolls plant<sup>-1</sup> and high seed cotton yield plant<sup>-1</sup>. The cluster two possessed only one genotype (BZU-75) which is characterized with relatively more number of bolls plant<sup>-1</sup>, relatively higher seed cotton yield plant<sup>-1</sup>, relatively higher micronaire value, and relatively longer staple length whereas cluster three also had one cotton genotype (SLH-4) with a characteristics feature of relatively lower in yield and micronaire value and relatively higher in staple length. The cluster four harbored total 10 cotton genotypes (BS-152, NS-161, Soybean, Tend-1, Tarzen-3, VH-282, CIM-602, Tarzen-402, NIB-1, JS-IV) which can be characterized by relatively lower in seed cotton yield plant<sup>-1</sup> and relatively higher in lint index and staple length. Cluster five had only one cotton genotype (AGC-777) and this could be characterized with relatively higher values for boll plant<sup>-1</sup>, micronaire value, GOT%, and staple length but comparatively lower values for seed cotton plant<sup>-1</sup>. Cluster six composed of two cotton genotypes (Soybean-202 and FH-118) which can be characterized as lower in bolls plant<sup>-1</sup> and seed cotton yield plant<sup>-1</sup>. Cluster seven also consisted of two genotypes such as Leader-1 and

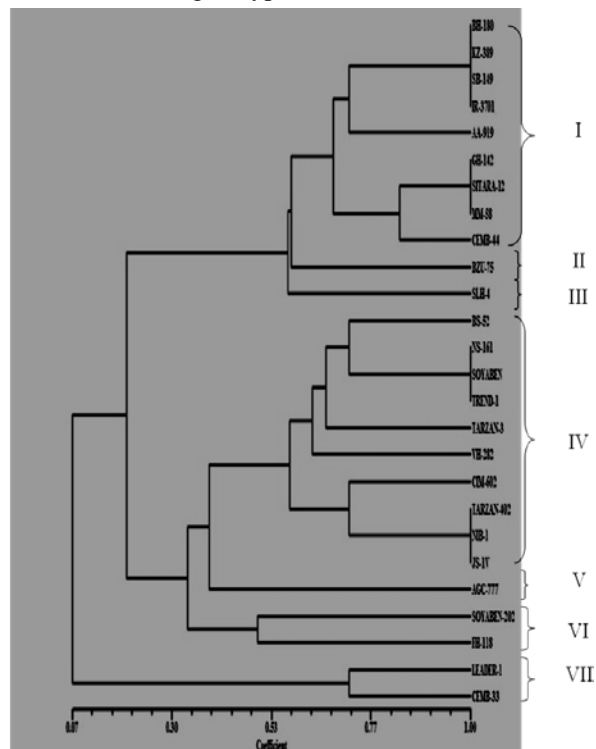


Fig. 1. Tree diagram of 26 Bt-cotton genotypes for 6 characters using hierarchical cluster analysis (Ward's Method)

CEMB-333 which having characteristics features of fewer number of bolls plant<sup>-1</sup>, low in seed cotton yield plant<sup>-1</sup>, low values for GOT%, lint index and staple length. On the basis of obtained results, it is suggested that the genotypes clustered together into group one, possessing desirable gene combinations for seed cotton yield plant<sup>-1</sup>, offering that these upland cotton genotypes could be used in future breeding programs in order to improve seed cotton yield. It has been suggested that genotypes grouped together into cluster two, six and seven should not be used in cotton breeding programs since the genotypes of these clusters contain undesirable gene recombination for seed cotton yield and its related traits and also lacking in suitable fiber traits. It is also recommended that hybridization program should be avoided between cluster one with clusters two, six and seven because later clusters do not possess reliable gene combinations for yield and fiber traits. Similarly to our results, Xian *et al.* (2012) also reported that genetic diversity analysis divided 38 cotton genotypes into two groups with similar genetic makeup, and the same breeding pedigree.

**Principal component analysis:** Principal component is one of the helpful statistical analyses which can be used to know the inter-relationship and variability of the genotypes. In this study, out of total six, only one principal component was extracted having Eigen value more than one. However, this principal component (PC1) contributed 94.20% of the total variability amongst the cotton genotypes assessed for various yield and fiber traits while remaining five principal components contributed only 5.80% towards the total diversity for this set of cotton genotypes. In total, first three principal components explained 99.00% variability, which is huge and can be utilized for further breeding programs in cotton. Yet, none of the trait positively associated with PC1. The positive and negative loading demonstrates the presence of positive and negative association tendency between the components and the variables. Therefore, the mentioned characters (**Table-3**) which load high positively and negatively contributed more to the diversity and they were the ones that most differentiated the clusters. Generally, it is customary to opt one variable from identified groups. Hence, from the second group micronaire value is best choice, which had the largest loading from component two, seed cotton yield plant<sup>-1</sup> for the third, lint index for the fourth group, bolls plant<sup>-1</sup> for the fifth group and GOT% for the sixth group. Similar to our findings, Saeed *et al.* (2014) also conducted principal component analysis in cotton genotypes for cotton curl viral disease, earliness and fiber quality traits and reported greater variability in first three principal components.

**Table 3. Vector loadings and explained percentage variance by the 6 PCs.**

Characters	Eigenvectors					
	PC1	PC2	PC3	PC4	PC5	PC6
Bolls plant <sup>-1</sup>	-0.4148	-0.1583	0.2764	-0.5159	0.5436	0.4061
Seed cotton yield plant <sup>-1</sup>	-0.4162	-0.1054	0.3351	-0.3665	-0.3796	-0.6519
Micronaire value	-0.3823	0.9120	0.0389	0.1256	0.0690	0.0058
GOT%	-0.4153	-0.2024	0.2679	0.3914	-0.5337	0.5262
Lint index	-0.4124	-0.2885	-0.0764	0.6120	0.4882	-0.3577
Staple length	-0.4075	-0.0885	-0.8557	-0.2366	-0.1806	0.0728
Eigenvalues	5.65329	0.20785	0.07708	0.04885	0.00813	0.004
Percent of variance	94.20	3.50	1.30	0.80	0.10	0.10
Cumulative percent of variance	94.20	97.70	99.00	99.80	99.90	100

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