Received: 12 Nov 2021 | Revised: 30 Nov 2021 | Accepted: 04 Dec 2021

Electronic Journal of Plant Breeding

Research Article

Genetic diversity analysis in *Bt* introgressed and non-*Bt* lines of upland cotton (*Gossypium hirsutum* L.)

Ashwini N R Samak[1*], S. Rajeswari[2], R. Ravikesavan[3], Subbalakshmi Lokanadhan [4], N. Ganapathy [5] and A. V. Kini[1]

[1]Indo-American Hybrid Seeds (I) Pvt Ltd, Bangalore, Karnataka, India
[2]Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[3]Department of Millets, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[4]Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[5]Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
*E-Mail: ashusamak@gmail.com

Abstract
Diversity analysis of *Bt* lines, a prerequisite to heterosis breeding, are rarely published. Diversity analysis of 140 cotton genotypes comprising of BGI, BGII and Non-*Bt* lines resulted in the grouping of genotypes into 20 clusters among which five had multiple genotypes and 15 others were solitary. Specific clustering of genotypes of BGI and BGII was not observed which got distributed across the clusters. The maximum and minimum inter-cluster distances were observed between two solitary clusters XVI and XVIII (159.8) as well as clusters VII and XI (6.4), respectively, reflecting diverse and close relationship among the *Bt* genotypes of those clusters. Two pairs of isogenic lines were located in the same clusters as they shared the same genetic background. The genetic background played important role in the diversity rather than the *Bt* status of genotype. Also, the maximum genetic diversity (64%) was arising from traits which were not related to *Bt* genes such as plant height, boll weight, and ginning out-turn when compared to the characters related to *Bt* gnes such as seed cotton yield and boll number (33%). In general, for seed cotton yield, BGI outperformed BGII lines. The study helped in developing high yielding hybrids through diverse parental line selections.

Key words: *Bt* cotton, Genetic diversity, Introgression, D^2 analysis

INTRODUCTION

Bt cotton is the most widely adopted genetically modified technology since 1996 when the first *Bt* cotton variety was approved for cultivation in the USA. By 2021, almost about 80-85% of the global cotton is under transgenic cotton cultivation, predominately with two events BGI (MON 531) and BGII (MON 15985). The rapid adoption of this technology is attributed to its undoubted safety and enormous benefit to farmers and the environment which is reflected in higher yield and less cost by protecting the crop from the damage caused by the bollworms without the need for the costly and environmentally hazardous chemical control. In India, the adoption of *Bt* cotton hybrids by the farmers have replaced the non-*Bt* cotton and *Bt* cotton hybrids that cover 98% of cultivated land. With this expansion of the technology, the hybrid development efforts have increased in *Bt* cotton.

Genetic diversity is the foundation for the development of new varieties/ hybrids. The precise information about the degree of relationship between different genotypes is very much essential for selecting diverse parents for hybridization programs. Genetic diversity between populations indicates the differences in gene frequencies. The cross involving genetically diverse parents is more likely to produce high heterotic effects as compared with lines that are more closely related to each other.

Multivariate analysis of the important yield-related characters and the genetic distances of the genotypes involved in the experiments provide valuable hints on possible genetic improvement schemes that can enhance the genetic worth of the population. Mahalanobis D^2 statistics has been utilized by several workers in a wide range of crop species including cotton to measure the genetic distance among their breeding material.

Cotton breeding programs across India are increasingly developing *Bt* cotton purelines to develop hybrids. Genetic diversity studies using *Bt* genotypes are rarely published, whereas, most of the published reports used non-*Bt* cotton (Malathi and Patil, 2019; and Nishanth *et al.,* 2015). The current study is aimed to assess the genetic diversity of cotton genotypes consisting of BGII, BGI and Non-*Bt* lines with estimations of plant characteristics for yield and its contributing characters.

MATERIALS AND METHODS

A set of diverse 140 cotton genotypes comprising of 72 BGII, 45 BGI and 23 Non- *Bt* genotypes developed by Indo-American Hybrid Seeds (I) Pvt. Ltd. (IAHS) were used to assess the genetic divergence among them. These materials were developed mostly by forwarding breeding methods using *Bt* cotton donor line (BGII) crossed with Non-*Bt* lines selected for desirable segregant that has *Bt* gene, to isolate *Bt* cotton pure lines and on a few occasions, isogenic lines were developed by backcross breeding methods. The experiment was conducted at Dharwad, Karnataka, India during the rainy season of 2018 in a randomized complete block design. Two rows of 10 dibbles with a spacing of 90 x 90 cm were planted with each genotype. Observations were recorded in five randomly selected plants in each genotype in each replication on seed cotton yield per plant, boll weight (g), ginning outturn, seed index (g), number of bolls per plant, plant height (cm), number of sympodia and monopodia per plant. The replicated data recorded was used for analysis using Mahalanobis (1936) D^2 statistics. Based on D^2 values the genotypes were grouped into different clusters by employing Tocher's method as outlined by Rao (1952).

Table 1.Clustering of genotypes by Mahalanobis D^2 analysis

Cluster	Genotypes	Number of genotypes
I	ICL- 35, ICL-103, ICL-14,ICL- 20, ICL-24, ICL-48, ICL-21, ICL-4, ICL-60, ICL-47, ICL-13, ICL-16, ICL-29, ICL-34, ICL-18, ICL-92, ICL-91, ICL-95, ICL-82, ICL-52, ICL-81, ICL-31, ICL-94, ICL-33, ICL-97, ICL-113, ICL-88,ICL-86, ICL-107, ICL-19, ICL-134 , ICL-104, ICL-129, ICL-57, ICL-17,ICL-44,ICL-127, ICL-50, ICL-76, ICL-40, ICL-89	41
II	ICL-78 BGI	1
III	ICL- 39 BGII	1
IV	ICL-65, ICL-137, ICL-110, ICL-109, ICL-9, ICL-119, ICL-111, ICL-126, ICL-87, ICL-26, ICL-100 , ICL-41, ICL-68, ICL-80, ICL-36, ICL-98, ICL-79, ICL-49, ICL-10, ICL-132,ICL- 45, ICL- 63, ICL-99, ICL-6, ICL-112	25
V	ICL-74, ICL-75, ICL-5, ICL-15, ICL-77 , ICL-85, ICL-101, ICL-93, ICL-105, ICL-30, ICL-12	11
VI	ICL-70, ICL-71, ICL-61,ICL-67, ICL-56, ICL-73, ICL-69, ICL-131, ICL- 28, ICL-66, ICL-139, ICL-54, ICL-37, ICL-11,ICL-124, ICL-83 , ICL-115, ICL-116, ICL-84, ICL-140, ICL-117, ICL-38, ICL-130, ICL-46, ICL-120, ICL-53, ICL-22, ICL-1, ICL-72, ICL-133, ICL- 118	31
VII	ICL-23 BGII	1
VIII	ICL-55, ICL- 123, ICL-59, ICL-32, ICL-06 , ICL-43,ICL- 2, ICL-128, ICL-64, ICL-42, ICL-122, ICL-121, ICL-102, ICL-8, ICL- 125,ICL-27,ICL- 62	17
IX	ICL-51BGII	1
X	ICL-7 BGII	1
XI	ICL-90 BGI	1
XII	ICL-25 BGII	1
XIII	ICL-138 N*Bt*	1
XIV	ICL- 136 N*Bt*	1
XV	ICL-3 BGII	1
XVI	ICL-135 N*Bt*	1
XVII	ICL-108 BGI	1
XVIII	ICL-58 BGII	1
XIX	ICL-114 BGI	1
XX	ICL-96 BGI	1

Genotypes with ICL-1 to 72 were BGII, ICL-73 to 117 were BGI and the rest were Non- Bt lines.

RESULTS AND DISCUSSION

Significant mean squares due to genotypes were observed for all the characters studied indicating diversity in the plant material under investigation.

All the 140 cotton genotypes were grouped in twenty distinct clusters based on yield and yield-related traits (**Table 1**). Among 20 clusters, 15 were solitary clusters and the rest of the clusters were having multiple genotypes. Cluster I contained the maximum number of genotypes i.e. 41 genotypes followed by cluster VI (31 genotypes), cluster IV (25 genotypes), cluster VIII (17 genotypes), and cluster V (11 genotypes). Clusters II, III and clusters IX to XX contained solitary genotypes. The majority of solitary clusters were *Bt* cotton lines (7 BGII lines and 5 BGI lines). A greater number of genotypes in a single cluster manifested that these genotypes were more closely related and had less genetic variation among them. The genotypes with shared pedigree were mostly clustered in the same group whereas the genotypes with different pedigree had enhanced diversity by being in different clusters. Mugheri (2015) reported a higher number of clusters while studying genetic diversity on *Bt*-cotton genotypes, indicating the greater genetic diversity for a variety of agronomic and yield traits based on cluster analysis in a group of *Bt* cotton genotypes. Interestingly, two pairs of isogenic lines (*Bt* and non-*Bt* genotypes) were included in the diversity analysis ICL-120 NBt and ICL-1 BGII as well as ICL-131NBt and ICL-54 BGII. All isogenic lines of Bt and non-Bt lines were clustered in the same cluster mainly due to sharing the same genetic background. It further implies that hybridization programs involving genotypes from the same cluster will be of little use in cotton improvement (Sharma *et al.,* 2016). Since the Bt and non-Bt genotypes were found in the same clusters, diversity is more driven by the genetic background than the Bt gene in the genotype. Most of the BGII lines (23 of 75) and BGI lines (15 of 45) were clustered in cluster I (23 lines BGII, 15 BGI and 3 Non-Bt lines). Likewise, in cluster V, seven out of 11 lines were BGI suggesting grouping of genotypes into different clusters did not follow any specific pattern (Sharma *et al.,* 2016). This can be due to the fact that most genotypes are being derived from forward breeding methods of *Bt* introgression and unidirectional selection.

Table 2. Cluster mean values for nine characters in 140 cotton genotypes.

Cluster	DFF	PH	MONO	SYM	NOB	BW	SI	SCY	GOT	Cluster Score	Cluster Rank
I	69 (4)	86.94 (17)	1.4 (6)	16.9 (15)	26.8 (12)	5.1 (9)	10.4(12)	1237 (11)	35.0 (12)	98	14
II	67 (3)	110.84 (7)	1.5 (5)	19.2 (3)	28 (10)	5.6 (5)	11.5 (4)	1260 (10)	30.1 (18)	67	4
III	71 (6)	90.67 (15)	1.2 (8)	15.3 (17)	31(7)	4.9 (11)	11.3 (5)	1599 (5)	30.5 (17)	97	13
IV	69 (4)	100.76 (10)	1.6 (4)	17.7 (8)	32.5 (6)	4.6 (13)	9.7(14)	1628 (4)	36.6 (10)	77	10
V	69 (4)	93.06 (13)	1.6 (4)	17.1 (13)	33.5 (5)	5.3 (7)	11.0 (7)	2105 (1)	36.6 (9)	67	5
VI	69 (4)	87.96 (16)	1.5 (5)	17.4 (11)	27.5 (11)	3.8 (17)	9.0(17)	930 (17)	34.8 (13)	115	18
VII	71 (6)	99.5 (11)	1.2 (8)	18.3 (6)	30.2 (8)	5 (10)	12.9 (3)	1192 (13)	29.2 (19)	89	11
VIII	69 (4)	73.51 (19)	1.5 (5)	15.0 (18)	24.8 (14)	5.1(9)	10.8 (8)	1188 (14)	30.9 (16)	111	16
IX	66 (2)	92.34 (14)	1.5 (5)	17 (14)	29.8 (9)	6.2 (4)	10.6 (9)	1398 (6)	41.8 (1)	67	6
X	71 (6)	113.5 (6)	1.3 (7)	17.5 (10)	21.5 (16)	4.8 (12)	10.6 (10)	1031(16)	27.3 (20)	109	15
XI	71 (6)	107.5 (8)	1.7 (3)	17.2 (12)	35.2(4)	5.2 (8)	13.1 (2)	1315 (7)	33.2 (15)	67	7
XII	70 (5)	105.67 (9)	1.7 (3)	17.7 (9)	24.8(13)	6.3 (3)	9.4(15)	1780 (3)	38.1 (6)	70	9
XIII	67 (3)	131.5 (2)	1.5 (5)	21.2 (2)	23.8 (15)	3.9 (16)	8.8 (18)	1089 (15)	35.7 (11)	90	12
XIV	65 (1)	118.5 (5)	1.6 (4)	18.5 (5)	40.5 (3)	4.6 (13)	10.5 (11)	1875 (2)	41.4 (2)	48	3
XV	69 (4)	121 (4)	1.8 (2)	18 (7)	35.2 (4)	6.4 (2)	13.6 (1)	1314 (8)	37.7 (8)	41	1
XVI	69 (4)	134.83 (1)	1 (9)	21.7 (1)	42 .0(2)	4.3 (15)	9.3 (16)	1193 (12)	40.1 (4)	67	8
XVII	67 (3)	126.38 (3)	1.9 (1)	19 (4)	45.7 (1)	5.5 (6)	11.3 (6)	1261 (9)	37.8 (7)	42	2
XVIII	70 (5)	54 (20)	1 (9)	13.8 (20)	16.2 (18)	4.5 (14)	6.8 (20)	618 (18)	34.7 (14)	142	20
XIX	67 (3)	95.84 (12)	1 (9)	15.8 (16)	10.8 (19)	3.4 (18)	8.7 (19)	262 (20)	41.2 (3)	123	19
XX	70 (5)	83.83(18)	1 (9)	14.7 (19)	17.2 (17)	6.7 (1)	10.1 (13)	548 (19)	39.8 (5)	111	17

DFF- Days to 50% flowering ; PH- Plant height (cm); MONO- Number of monopodia/plant; SYM- Number of sympodia/plant; NOB- Number of boll/plant; BW- Boll weight (g) ; SCY- Seed cotton Yield (kg/ha) ; SI- Seed Index (g) ; GOT- Ginning outturn (%) Figures in parenthesis indicates the trait rank

Analysis of cluster means revealed the relative contribution of different traits to the total divergence by the different clusters. The highest cluster means for the character was given the first rank and the next cluster possessing the next best means were given 2nd, 3rd and so on up to 20th rank for all the traits. Finally, the clusters are ranked based on the overall score obtained from nine characters. The lowest scoring cluster was given the first rank, and the next cluster possessing the score above the previous ones were given 2nd, 3rd and so on up to 20th rank. The utilization of low ranked clusters (high cluster means) in a breeding program is expected to yield desirable lines in the advanced generation of selection (**Table 2**). In the present investigation, based on morphological and yield contributing characters, it was observed that genotypes grouped under cluster XV were ranked first by having ten characters (1 to 20 scores) in the desirable direction followed by genotypes under cluster XVII with nine characters. Cluster V had the highest mean seed cotton yield and ranked fifth overall which is having *Bt* cotton lines. Among the top four clusters, three were solitary with *Bt* genotype. Similar works were reported by Malathi and Patil (2019) and Nishanth *et al.* (2015).

Intra and inter-cluster distance (D^2) values for 140 cotton genotypes grouped into 20 clusters are presented in **Table 3**. The maximum inter-cluster distance (159.8) was observed between two solitary clusters XVI (Non -*Bt*) and XVIII (BGII) followed by another pair of the solitary clusters (147.9) between XVII (BGI) and XVIII (BGII). This indicated that the genotypes in these clusters were far diverse than those of other clusters. The cross between the genotypes of these two divergent clusters will give heterotic hybrids with high yield and also the resultant segregants from the crosses would be promising. The minimum inter-cluster distance was observed between clusters VII and XI (6.4) suggesting that genotypes within these clusters were not genetically diverse. The results were in agreement with Akter *et al.* (2019) and Kavithamani and Amalabalu, (2017). The top three highest intra- cluster distance was observed in cluster VIII (28.8), cluster VI (24.8) and cluster V (19.7). Among these clusters, VIII and V are composed of *Bt* cotton lines.

The characters contributing maximum to the divergence are given greater emphasis for deciding the cluster for further selection and the choice of parents for hybridization (Siddique *et al*., 2010). The relative contribution of nine characters towards the total divergence is presented in **Table 4**. Among nine characters, plant height was the largest contributor (24.38%) towards divergence followed by seed cotton yield (22.27%) and boll weight (17.97%).

Table 3. Intra and Inter cluster distance values for 140 cotton genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX
I	**13.9**	21.8	19.7	25.3	32.9	28.1	25.6	27	18.2	28.8	29.6	30.8	56.2	54.6	40.3	74.2	53.0	46.6	69.3	38.0
II		**0**	15.2	26.1	34.3	41	11.3	38.3	27.7	7.86	13.2	28	38.5	44	16.3	54.8	28.0	88.7	92.4	56.0
III			**0**	22.1	19.9	38.7	7.98	28.1	33.2	19.2	12.6	39.7	62.4	43.6	32.2	72	44.0	84.3	105	73.0
IV				**17.2**	25.5	35.3	34.4	50	24	33	29.8	28.9	37.5	24.7	37.1	44.3	36.0	82.6	78.3	69.0
V					**19.7**	56.9	37.8	51	31.3	48	33.4	31.4	71.4	37.5	42.8	76.2	53.0	102	123	86.0
VI						**24.8**	41.3	45.1	39.2	41	46.4	60.2	48.5	59.8	64.8	67.6	64.0	49.9	48.7	59.0
VII							**0**	32.7	44.7	15.5	6.43	58.2	62.2	56.1	25.9	72.8	42.0	96.5	102	76.0
VIII								**28.8**	43.5	44.2	44.4	58.7	96.7	94.4	66.4	123	88.0	51.1	100	56.0
IX									**0**	43.6	38.9	13.7	53	39.2	29.9	59.1	39.0	57.1	69.9	24.0
X										**0**	23.3	39.4	31.9	56.2	32.6	59.9	43.0	89.4	78.1	62.0
XI											**0**	52.4	57.1	37.6	11	55.6	24.0	114	105	80.0
XII												**0**	51.1	47.4	39.4	69.6	52.0	80.1	99.2	42.0
XIII													**0**	31.1	52.8	21.2	41.0	118	47.4	88.0
XIV														**0**	33.6	16.9	20.0	146	95.7	110.0
XV															**0**	45.8	13.0	134	110	68.0
XVI																**0**	18.0	160	86.7	113.0
XVII																	**0**	148	112	90.0
XVIII																		**0**	64.9	35.0
XIX																			**0**	62.0
XX																				**0**

Table 4. Contribution of traits towards divergence in cotton genotypes

S. No.	Trait	Contribution (%)
1	Days to 50% flowering	0.46
2	Plant height	24.38
3	Number of monopodia	0.68
4	Number of sympodia	0.4
5	Number of bolls	10.85
6	Boll weight	17.97
7	Seed Index	6.28
8	Seed cotton yield	22.27
9	Ginning out turn	16.19

Table 5. Performance of transgenic and non-transgenic cotton genotypes for yield and its contributing characters.

Transgenic status	BG II			BG I			Non-Bt		
Number of lines	72			45			23		
Characters	Mean	Range	S.E	Mean	Range	S.E	Mean	Range	S.E
Days to 50% flowering	69	66-74	0.25	69	66-73	0.3	68	64- 72	0.38
Plant height (cm)	88.2	51.5-121	1.64	91.51	65.8 -126.4	2.02	95.6	54.7- 134.8	4.35
Number of monopodia /plant	1.5	1- 2.7	0.05	1.4	1-2.2	0.05	1.5	1 -2.5	0.08
Number of sympodia/ plant	17	13– 22	0.22	17.19	15 - 20	0.22	17	13 - 22	0.48
Number of bolls/ plant	28.5	11.8- 45	0.7	30.0	10.8- 45.6	1.12	19	16.5 -25	1.64
Boll weight (g)	5.1	3.04-6.4	0.1	4.9	2.9 - 6.7	0.12	4.5	3.65 - 4.9	0.14
Seed index (g)	9.8	6.8 - 13.6	0.17	10.4	8.2 - 13.1	0.16	10.3	8.7- 13.9	0.29
Seed cotton yield (kg/ha)	1293	993 –2222	44.41	1399	262 -2226	77.0	951	502 -1075	66.26
Ginning out turn (%)	34.2	22.9 - 41.7	0.45	36.1	30.1 -41.9	0.46	34.9	25.9 - 39.4	0.79

A similar result was reported by Khan *et al*. (2015) in transgenic cotton. Boll number and the seed cotton yield which were directly related to the *Bt* gene together contributed 33 per cent diversity whereas, plant height, ginning outturn, seed index and boll weight which are not influenced by the *Bt* gene in the genotype together contributed 64 per cent to the diversity. The above results implied that to select genetically diverse parents, selection strategy should be based on the traits like seed cotton yield, plant height and boll weight. De *et al*. (1988) stated that traits contributing maximum towards the D^2 values needed to be given more emphasis for deciding the clusters to be taken for choice of parents for hybridization. The *per se* performance of transgenic and non-transgenic lines was also compared (**Table 5**) and the mean performance of seed cotton yield and its contributing characters were higher in BG I group as compared to the BGII group. Better bollworm control in *Bt* genotypes leads to greater retention of early-formed bolls which transformed to higher seed cotton yield (Hebbar *et al*., 2007). In grouping genotypes, transgenic and non-transgenic lines were spread in all clusters.

To develop superior parents, understanding the genetic diversity of the genetic materials is the basic step in the breeding program. In the current study, highly significant differences were observed among *Bt* and Non-*Bt* cotton genotypes evaluated for all the nine studied traits. *Bt* cotton lines were significantly higher yielding than non-*Bt* genotypes. Irrespective of *Bt* or non-*Bt*, genotypes were distributed in 20 clusters (5 with multiple genotypes and 15 with solitary) and there were no specific clusters for *Bt* genotypes. This could be because the *Bt* unrelated traits (plant height, ginning outturn and boll weight) together contributed the maximum to the observed diversity. The majority of the solitary clusters were with *Bt* genotypes (7 BGI and 5 BG II) indicating the forward breeding of the Bt genotypes has created diversity. The study helped to select the diverse BGI and BGII parents to develop hybrids.

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