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Parental Diversity and Earliness-Seed-Lint Index of Twelve Cotton (*Gossypium hirsutum* L.) Genotypes

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Abstract: The study aimed to assess the genetic diversity and relationships among 12 genotypes using RAPD markers. Molecular variance within and between the populations of parental genotypes accounted for 93% and 7%, respectively. The unweighted neighbor-joining tree, principal coordinate analysis, and heatmap clustered the 12 parental genotypes into three major groups. The population structure model divided the population into two groups, with parents BC-119 and RA-2 being pure. Parents BC-119 and P1(1) required the fewest days for 80% boll opening (166.97 and 168.78, respectively). Parents RA-2 and P1(1) also achieved the highest seed cotton yield per plant (68.47 g and 68.09 g, respectively). Parent BC-119 exhibited strong performance in traits including the number of secondary fruiting branches (10.64 per plant), lint index (5.46), and ginning out-turn (41.26%). The highest value of Bartlett's earliness index was 1.11. CB-15 and CB-17 genotypes produced the highest seed index, while genotype P1(1) recorded the lowest seed index. Parents BC-119 and CB-17 yielded the highest lint index (>5.30 g), whereas P3(8) produced the lowest lint index (2.93 g). Results suggested that molecular marker data are useful in determining the diversity and relatedness of parents, which could be used to identify exceptional lines for future breeding programs.

1 Introduction

Cotton (*Gossypium hirsutum* L.) is an important natural fiber crop and the second most significant oil-producing crop worldwide (Upadhyay et al 2019). It plays a crucial role in the textile industry and is valued at 600 billion US dollars in the global economy (Razzaq et al 2020). In Bangladesh, cotton stands as the most vital cash crop after jute; in 2022-23, the area and production of cotton were 45,000 hectares and 153,000 bales, respectively. Due to a nearly three percent decrease in harvested area, world cotton production is expected to be slightly lower in 2022–2023. Because of the narrow genetic base and the absence of innovative techniques, the genetic diversity of Gossypium species, along with fiber yield and quality, has been declining globally for many years in cultivated cotton (Mohamed et al 2003).

To maximize breeding potential and conserve genetic resources, information regarding the distribution and degree of genetic diversity, as well as the relationships between the genetic materials, serves as a component for parent selection and estimating the extent of heredity, dissimilarity, and hybrid vigor (Upadhyay et al 2019). Furthermore, to develop high-yielding varieties in any crop, it is important to have genetic diversity in the breeding materials that the plant breeder could exploit in the selection processes of the breeding programs (Solanki et al 2014). Using genetically diverse parents is one of the most crucial genetic factors (Sawarkar et al 2015). Conventional genetic improvement breeding has limitations, primarily because polygenic variables, such as fiber yield, quality, productivity, biotic and abiotic stress tolerance, and challenging growth environments, are poorly understood.

One of the significant advancements is the use of genetic markers in plant breeding, which helps quick identification of polymorphism in the DNA of the target materials (Sabev et al 2020). Cotton genome study has used various PCR-based DNA markers, for example, RAPD, SCAR, SRAP, AFLP, and RGA (Zhang et al 2008). Among them, RAPD markers are ideally suited for determining similarities and differences across various cotton cultivars since they are relatively easy to use, less expensive and dependable. They are primarily used to promote parental diversity, allowing for the production of a strong hybrid. Therefore, the current experiment was designed to investigate the genotypic divergence of 12 parental genotypes of *Gossypium hirsutum* using eight RAPD markers and the earliness-seed-lint index, providing essential information for cotton improvement.

2 Materials and Methods

2.1 Experimental materials

Twelve parental genotypes, namely M1, RA-2, P1(1), P3(8), BC-119, BC-120A, BC-120B, CB-9, CB-12, CB-14, CB-15, and CB-17, were used as experimental materials (**Table 1**). The seeds of these genotypes were collected from the Cotton Gene Bank of the Cotton Development Board (CDB) in Mahigonj, Rangpur. The seeds were then sown in the Research and Seed Multiplication Farm of CDB at Sreepur, Gazipur, in a plot measuring 4 x 3.5 m², with a spacing of 90 cm x 45 cm. The recommended dose of fertilizers was applied in the experimental field according to CDB standards. The field experiment used a Randomized Complete Block (RCB) Design with three replications.

Table 1. Sources and salient features of parental genotypes used in the study

Sl. No.	Genotypes	Sources	Salient features
1	CDB Tula M1	CDB released mutant variety	BP (30-40), early type, DM (150-160), drought tolerant, GOT-42%, white lint
2	RA-2	CDB germplasm	BP (23), DM (170-180), GOT-32%, long fibre, white lint
3	P1(1)	Advanced line	BP (32), short duration (150-160), GOT-33%, white lint
4	P3(8)	Advanced line	BP (22), Short duration (160-170), GOT-23%, white lint
5	BC-119	CDB germplasm	Okra leaf, purple colour, GOT-41%, brown lint
6	BC-120A	CDB germplasm	Green colour plants, GOT-34%, white lint
7	BC-120B	CDB germplasm	Purple colour plants, GOT-29%, greenish lint
8	CB-9	CDB released variety (SI 4 /91/646: (released in 2005)	BP (44), late type, DM (190-200), Jassid tolerant, bollworm susceptible, white lint
9	CB-12	CDB variety (released in 2013)	BP (40), DM (170-180), bollworm tolerant, GOT-40%, white lint, well accepted by farmers
10	CB-14	CDB variety (released in 2013)	BP (45), DM (175-185), Jassid and aphid tolerant, high yield, GOT-38%, white lint, well accepted by farmers
11	CB-15	CDB variety (released in 2016)	BP (40), DM (180-190), uniform ball splitting, GOT-42%, white lint, well accepted by farmers
12	CB-17	CDB variety (released in 2018)	BP (40), DM (180-190), white lint

CDB= Cotton Development Board; BP= balls per plant; DM= days to maturity, GOT= ginning out turns

2.2 Molecular Analysis

Leaf samples collected from freshly germinated seedlings were used to extract DNA using the CTAB (Cetyl Trimethyl Ammonium Bromide) method. RAPD amplification was performed using the PCR ingredients (**Table 2**) and maintaining the temperature, time, and number of PCR cycles (**Table 3**). The PCR amplified products were segregated on agarose gel (0.8%) using TAE buffer (1X) and ethidium bromide (0.5 µl) and visualized in the Bio-Rad Gel Doc EZ System.

Table 2. PCR ingredients for RAPD markers (for 14µl)

Ingredients name	Volume required (µl)
H_2O	3.5
Master mix	7.5
Primer	1
DNA	2

2.3 Agronomical parameters

Data were collected on agronomic characters *viz*., the height of the plant at harvest (cm), the number of nodes for bearing first fruit, monopodial branch (no.), sympodial branch (no.), secondary fruiting branch (no.), bolls per plant (no.), % boll retention, %1st picking, days to squaring, first flowering, first boll opening (days), 50% boll opening (days), 80% boll opening (days), single boll weight (g), seed cotton yield (g/plant), lint yield (g/plant), GOT (ginning out turn %), earliness index (Bartlett 1937), seed index (g) and lint index (Khan et al 2010), to determine the agronomical performance of the parents.

2.4 Data Analysis

Means of the data collected across seasons were subjected to analysis of variance (ANOVA) using Statistical Tool for Agricultural Research (STAR) Version 2.0.1, and significant means were compared using least significant differences at $P \leq 0.05$ and $P \leq 0.01$. From the resulting gel image, the presence (1) or absence (0) was scored and recorded into the data sheet. Data were uploaded as a binary matrix (one for each marker). Analysis of molecular variances was performed by GenAlEx 6.5 (Peakall and Smouse 2012) to determine genetic variances both between and within genotypic groups. The dendrogram was constructed using the neighbor-joining algorithm from the distance matrix with the help of computer-based software DARWIN 6.0. Principal coordinate analysis (PCoA) was done using the statistical package PAST. Heatmap was created using a pairwise distance matrix by the HEATMAPPER program (Babicki et al 2016). The PIC value for each primer was calculated as PIC 2fi(1-fi), where PIC is the polymorphism information content of the marker, fi is the frequency of the marker fragments that were present, and 1-fi is the frequency of the absent marker fragments. The population structure was done with the help of STRUCTURE Software Ver 2.3.4 (Pritchard et al 2000).

Table 3. RAPD markers, their sequences and PCR cycles used in the genetic diversity study of 12 parental genotypes of cotton

Name of primers	Sequences (5'-3')	Steps	Temperature (°C)	Duration	Cycles
OPA 7	GAAACGGGTG	1	94	2 min	42x
		3	42	30 sec	
OPA 8	GTGACGTAGG	1	94	2 min	42x
		3	42	30 sec	
OPA 9	GGGTAACGCC	1	94	2 min	42x
		3	42	30 sec	
OPA 10	GTGATCGCAG	1	94	2 min	42x
		3	42	30 sec	
OPC 3	GGGGGTCTTT	1	94	2 min	42x
		3	42	30 sec	
OPD 5	TGAGCGGACA	1	94	2 min	42x
		3	42	30 sec	
OPD 17	TTTCCCACGG	1	94	2 min	42x
		3	42	30 sec	
OPD 20	ACCCGGTCAC	1	94	2 min	42x
		3	42	30 sec	

3 Results and Discussion

3.1 Analysis of molecular variance (AMOVA)

Analysis of Molecular Variance (AMOVA) is used to identify variation in a genetic population using DNA markers (Excoffier et al 1992). For this experiment, we used eight RAPD markers for twelve parental cotton genotypes. The analysis of molecular variance helps distinguish genetic variation between the green and colored genotypes of cotton. AMOVA based on RAPD marker scores showed that 7% of the total genetic variation occurred within populations, while 93% was found among the populations (**Fig 1**). Variation among populations refers to differences between two or more populations. Within the population, there was a variation of about 93%. Cross-pollination occurs in cotton, comprising 5-50% of the cotton crop, despite cotton being a self-pollinated crop (Stephens and Finkner 1953). AMOVA was also computed by Baran et al (2023), who reported that differences within the population account for 69% of the genetic variance in cotton.

3.2 Principal Coordinate Analysis (PCO)

PCO was performed to assess the consistency of differentiation among populations identified by cluster analysis and to provide a three-dimensional representation of the comparative genetic distances between genotypes. It was conducted based on genetic dissimilarities from RAPD data presented across four axes (**Fig 2**). In the upper right axis, two genotypes, BC-120B and P1(1), which were not closely related, indicated high genetic dissimilarity. In the upper left axis, five genotypes —M1, P3(8), BC-120A, CB-9, and CB-17 —were clustered closely together, indicating lower genetic dissimilarity. The bottom left axis features two genotypes, BC-119 and CB-14, which are not closely related, indicating a higher degree of genetic dissimilarity. In the bottom right axis, three genotypes, RA-2, CB-12, and CB-15, with greater distances indicated higher dissimilarity. PCO was performed to assess the consistency of differentiation among populations identified by cluster analysis and to provide a three-dimensional representation of the comparative genetic distances between genotypes. It was conducted based on genetic dissimilarities from RAPD data presented in four axes (**Fig 2**). In the upper right axis, two genotypes, BC-120B and P1(1), that were not closely related indicated high genetic dissimilarity. In the upper left axis, five genotypes were represented, named M1, P3(8), BC-120A, CB-9, and CB-17, which were closely grouped, indicating lower genetic dissimilarity. The bottom left axis, which displays two genotypes named BC-119 and CB-14 that are not closely related, indicating higher genetic dissimilarity. In the bottom right axis, three genotypes, RA-2, CB-12, and CB-15, with higher distances indicated higher dissimilarity.

3.3 Neighbor-joining tree

The neighbor-joining tree is a widely used method for constructing phylogenetic trees (Saitou and Nei 1987). We constructed a dendrogram that showed three major clusters among 12 cotton genotypes using the UPGMA (Unweighted Pair Group Method with Arithmetic Average) method, indicating the existence of hereditary dissimilarity between the genotypes (**Fig 3**). Red indicates cluster A, which is subdivided into two sub-clusters. One sub-cluster contained the genotypes 1 (M1), 5 (BC-119), 10 (CB-14), and 12 (CB-17), while another sub-cluster contained two genotypes, 8 (CB-9) and 6 (BC-120A). Blue indicates cluster B, containing four genotypes: 2 (RA-2), 3 [P1(1)], 9 (CB-12), and 11 (CB-15). Black indicates cluster C, containing two genotypes, 4 [P3(8)] and 7 (BC-120B). The members of each section share a set of physical and genetic characteristics. As a result, using parents from a diverse group, a strong heterotic couple can be created.

3.4 Heatmap: Visualizing Genetic Importance of Traits

A heatmap was created to visualize complex data in a graphical format using a color matrix, making it easy to understand at a glance. The heatmap aids in evaluating the performance of the parental genotypes of cotton. It was constructed using dissimilarity matrix data. Large numerical values are represented by dark squares and smaller numerical values by light squares, according to Tiessen et al (2017). The heatmap illustrated the correlation between the genotypes (**Fig 4**). In the heatmap, we observed that parents 1 (M1), 5 (BC-119), 10 (CB-14), and 12 (CB-17) exhibit lower genetic distances from one another. Parent 2 (RA-2) had a higher genetic distance from parents 3 [P1 (1)], 7 (BC-120B), 9 (CB-12), and 11 (CB-15), indicating greater genetic dissimilarity with a value of 1.4234. Parent 5 (BC-119) showed dissimilarity with parents 2 (RA-2), 3 [P1 (1)], 4 [P3 (8)], 6 (BC-120A), 7 (BC-120B), and 8 (CB-9). Furthermore, parents 6 (BC-120A) and 8 (CB-9) exhibited the greatest dissimilarity from the other parents.

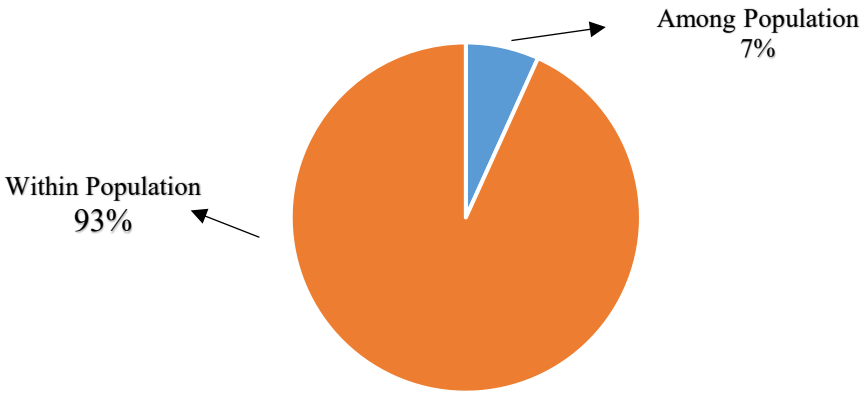

Fig 1. AMOVA showing variance percentages for the twelve parental cotton genotypes

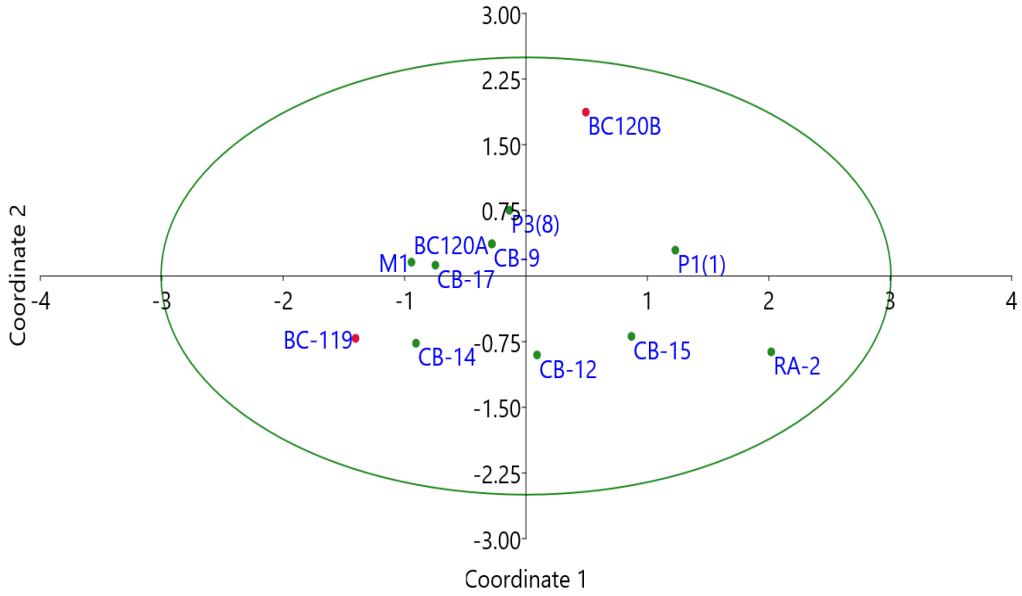

Fig 2. Principal Coordinate Analysis (PCO) of 12 parental cotton genotypes using eight RAPD markers. Green points indicate green cotton plants and red points indicate purple cotton plants

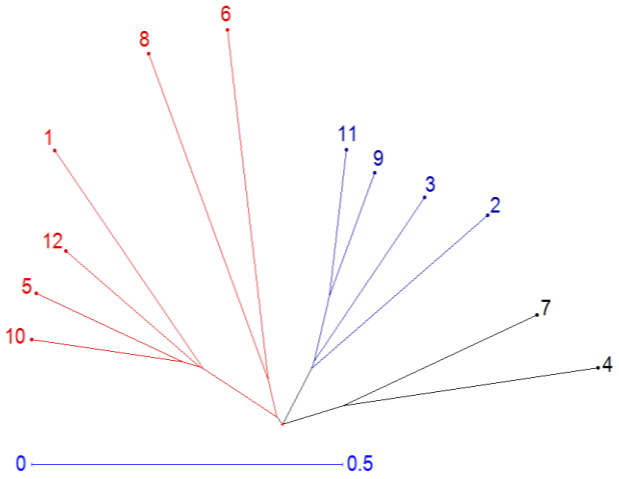

Fig 3. A neighbor joining tree from Darwin based on RAPD data constructed for the twelve parental cotton genotypes (1= M1, 2= RA 2, 3= P1(1), 4= P3(8), 5= BC 119, 6= BC 120A, 7= BC 120B, 8= CB 9, 9= CB 12, 10= CB 14, 11= CB 15 and 12= CB 17)

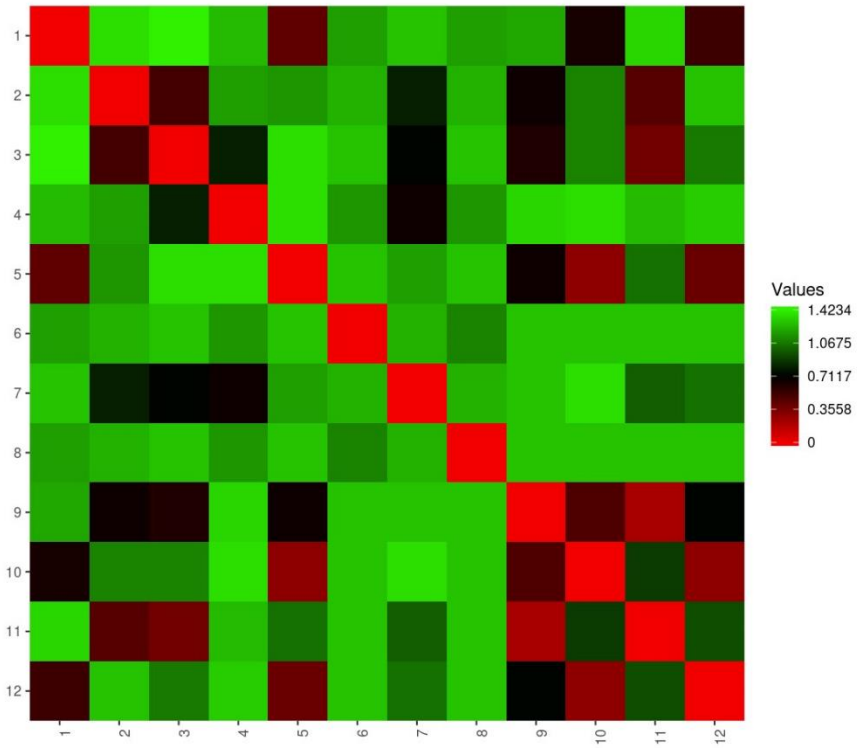

Fig 4. Heatmap of pairwise correlation between the twelve cotton parental genotypes when bright red colour indicates strong positive correlation or lower genetic distance whereas bright green indicates a strong negative correlation or higher dissimilarity between genotypes (1= M1, 2= RA 2, 3= P1(1), 4= P3(8), 5= BC 119, 6= BC 120A, 7= BC 120B, 8= CB 9, 9= CB 12, 10= CB 14, 11= CB 15, 12= CB 17)

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Parent 12 (CB-17) displayed dissimilarity with parents 2 (RA-2), 3 [P1 (1)], 4 [P3 (8)], 6 (BC-120A), and 8 (CB-9). No genetic distance (0 value) was observed for intra-parental values, and the least dissimilarity was found across the heatmap.

3.5 Polymorphic information of the eight RAPD primers

Polymorphism Information Content (PIC) measures the amount of polymorphism produced by the molecular or DNA marker. Genetic variation within and among the population was evaluated using PIC value as an index (Botstein et al 1980). Therefore, the genetic diversity of germplasm at each locus is determined using polymorphic information content and markers can be informative when the range is between 0-0.5.

In this experiment, we measured the PIC values of 12 parental cotton genotypes using eight RAPD markers (**Fig 5**). The PIC value ranges from 0.0058 for the primers OPC 3 and OPD 17 to 0.1342 for the primer OPA 9. The autogamous nature and narrow genetic base may account for the low polymorphism observed in cotton genotypes. A high PIC value indicates greater genetic variability. In our experiment, the OPA 9 primer displayed the highest PIC value (0.1342), which is significant in other genetic studies, followed by the primer OPA 10 (0.1275), OPD 20 (0.1252), and OPA 7 (0.1238).

3.6 Population structure analysis

Genetic relationships based on population structure are crucial for establishing the appropriate scale and subunits for conservation management. Population structures primarily reflect the genetic differentiation among individuals within a population. In the population structure analysis, the highest ΔK value indicated a strong peak for the 12 parental genotypes across two populations, where K=2 signifies that the optimal number of clusters was 2 (**Fig 6**). The population structure of the studied plants exhibited admixture in the two groups (red and green populations). The cluster membership probabilities of each individual from the 12 genotypes are displayed in **Fig 7**. A proportion of each population with values >0.80 was deemed pure, while populations with lower values were regarded as admixed. The green population comprised 5 genotypes: 1(M1), 4[P3(8)], 6(BC-119), 8(CB-9), and 12(CB-17), all above 0.60, but only genotype 6(BC-119) exceeded 0.80, indicating purity. From the perspective of the red population, it included one genotype, 2(RA-2), which was less pure. The remaining genotypes were admixed.

3.7 Agronomic performance of the parental genotypes

The agronomic performance of genotypes is considered the primary selection criterion for choosing parents in a hybridization program to create promising hybrids. The parents with superior performance result from good hybrids (Gilbert 1958). **Table 4** presents the average agronomic performance of 12 parental cotton genotypes.

The plant height is genetically determined; however, it is also influenced by environmental factors and nutritional stress (Ahmad et al 2009). The variation in plant height among the parents was not significant, ranging from 67.71 cm to 87.41 cm (**Table 4**). The minimum plant height observed was 67.71 cm in parent M1, while the highest mean value of 87.41 cm was found in parent BC-119. One of the early-maturing types establishes the first effective boll on lower sympodial branches. The parents in this study showed significant differences ($P<0.05$) in the number of nodes producing the first fruiting branch (**Table 4**), with values ranging from 4.33 to 6.36. Parent P3(8) had the lowest value of 4.33, followed by BC-119 and M1, with respective values of 5.11 and 5.22. The other parents produced their first fruiting branch nodes, which ranged from 5.42 to 6.36. Parent CB-15 had the highest mean value of 6.36, followed by parents CB-14 and RA-2, both with values of 6.17.

The number of monopodial branches per plant in 12 cotton genotypes ranges from 1.11 to 2.31 (**Table 4**). The parent M1 exhibited the lowest estimate (1.11), followed by CB-15 (1.33) for the studied trait. The highest number of monopodial branches per plant was observed in the parent BC-119 (2.31), followed by P3(8) and CB-17 with respective values of 2.17 and 2.11. The sympodial branch, which refers to cotton's fruit and boll-bearing branches, strongly predicts a plant's ability to produce cotton fiber. The number of sympodial branches per plant ranges from 11.94 to 15.50 for parents (**Table 4**). The parent P3(8) produced the highest number

(15.50) of sympodial branches per plant, followed by BC-119 with a value of 14.78. The lowest number of sympodial branches per plant was observed in M1, followed by RA-2, with values of 11.94 and 12.03, respectively. Secondary sympodial branches are sometimes produced from productive monopodial branches (McGarry et al 2016). The number of secondary fruiting branches per plant in the parental genotypes ranges

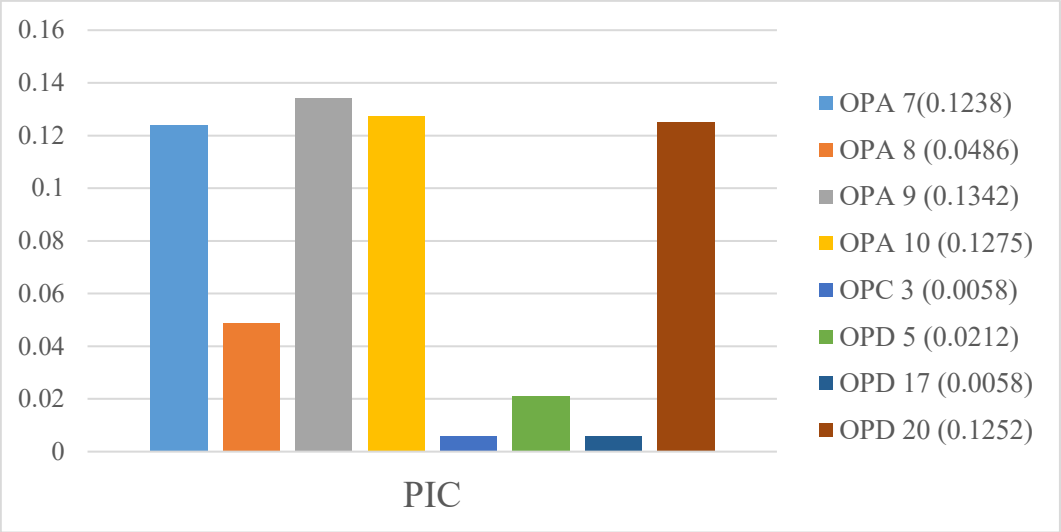

Fig 5. Polymorphic fragment generated PIC values by eight RAPD markers in 12 parental cotton genotypes

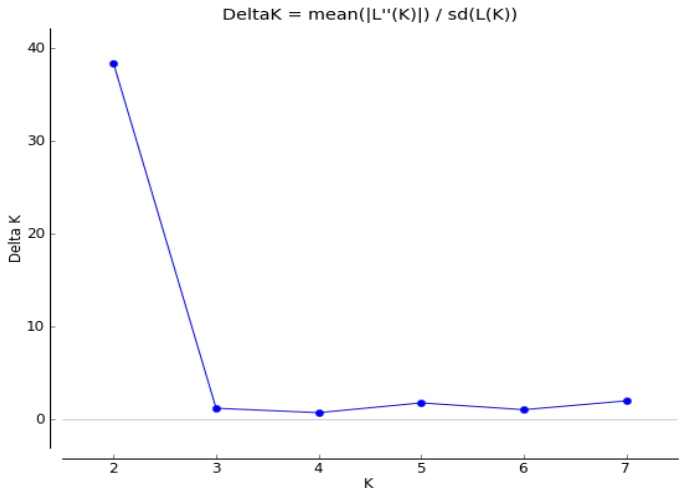

Fig 6. Population structure through LnP(D) derived ΔK values reached a maximum at K=2

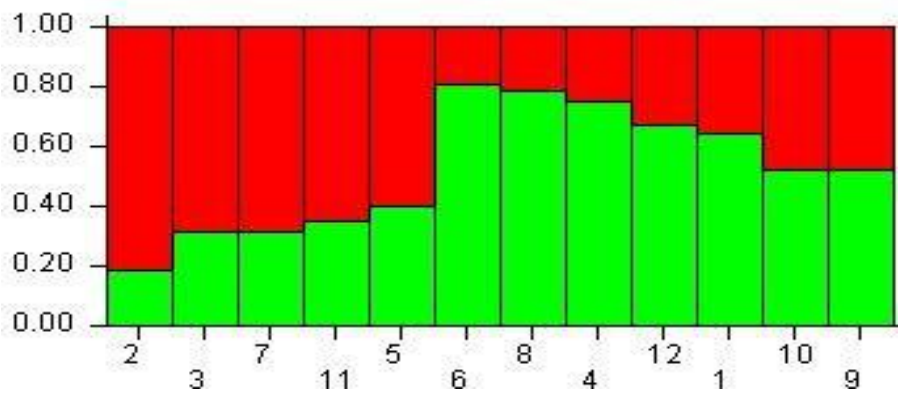

Fig 7. Model based population structure of 12 cotton parental genotypes by eight RAPD primers (1= M1, 2= RA 2, 3= P1(1), 4= P3(8), 5= BC 119, 6= BC 120A, 7= BC 120B, 8= CB 9, 9= CB 12, 10= CB 14, 11= CB 15, 12= CB 17)

from 2.89 to 10.64 (**Table 4**). The parent BC-119 exhibited the highest mean performance (10.64), followed by CB-9 (9.39) for the studied trait. The lowest mean performance was observed for the parent M1 (2.89), followed by RA-2 (5.86).

The cotton variety that requires a minimum of one day to set the first square will also set and open bolls earlier. Significant variation ($P < 0.01$) was observed in the days to squaring among the parents (**Table 4**). The mean value for this trait ranged from 55.25 to 58.14 days. Parent P1(1) took a minimum of 55.25 days to square, while the maximum days to square, 58.14, was observed for the parent BC-120 B. Days to flowering is considered a major selection index that influences boll opening to determine the earliness of the genotypes; however, it does not directly affect seed cotton yield per plant. The mean variation among parents for days to the first flowering ranged from 68.33 to 73.08 days (**Table 4**). Parents displayed no significant variation in the number of days to the first flower. Parent P1(1) began flowering at 68.33 days, whereas parent BC-120A started at 73.08 days.

Boll opening is a crucial characteristic for quantifying earliness in cotton varieties. In the case of the first boll opening, a highly significant variation ($p<0.01$) was observed among the cotton genotypes. The mean value ranged from 131.25 to 139.03 (**Table 4**). BC-119 and P1(1) took the fewest days (131.25 and 132.58, respectively) for the first boll opening. For days to 50% boll opening, significant variation ($p<0.01$) was noted among the parental genotypes (**Table 4**). The mean performance spanned from 156.47 to 177.83 days for this trait. The parent BC-119 required the fewest days (156.47), while CB-14 needed the most (177.83) for 50% boll opening. Parental genotypes exhibited significant variation for 80% boll opening (days). The average performance of the genotypes ranged from 166.97 to 195.00 days (**Table 4**). The parents BC-119 and P1(1) required the fewest days (166.97 and 168.78, respectively) for 80% boll opening, whereas parent BC-120B reached its 80% boll opening in 195.00 days.

The cotton bolls are essential components of seed cotton yield and fiber quality. Lint yield is mainly influenced by the number of bolls per plant, seeds per boll, and lint mass in the seed. The mean performance for bolls per plant varies from 12.67 to 22.36 for parents (**Table 4**). Among 13 parents, parent P1(1) produced the highest number of bolls per plant at 22.36, while parent P3(8) had the lowest at 12.67. For percent boll retention, significant variation was observed among the parental genotypes, with percentages ranging from 56.40 to 80.34. M1 showed the highest boll retention of 80.34 per plant, followed closely by P1(1) at 79.63. The lowest boll retention percentage of 56.40 was found in parent P3(8).

In the case of the percentage of first picking, significant variation was observed among the parents. The mean value of this trait ranged from 10.92 to 56.51 (**Table 4**). The parent RA-2 exhibited the highest mean value of 56.51, followed closely by parent P1(1) with a value of 56.18. The lowest value, 10.92, was recorded for parent P3(8). Boll weight is a key yield-contributing characteristic that directly impacts seed cotton yield. Therefore, it is important to consider boll weight carefully during selection. Significant variation was noted in boll weight, with values ranging from 3.02 to 4.79 g (**Table 4**). Parent CB-12 produced the heaviest bolls, weighing 4.79 g, followed by parent CB-15, which had a boll weight of 4.75 g compared to all other parents. The minimum boll weight recorded was 3.02 g for parent BC-119 and 3.13 g for parent BC-120A, respectively.

Final yield per hectare will be higher if each plant produces more seed cotton (Ahmad et al 2009). Significant variations ($P<0.01$) were observed among the parents for seed cotton yield per plant (**Table 4**). The average value ranges from 19.62 to 68.47 g. The parents that produced the maximum seed cotton were RA-2 and P1(1) (68.47 and 68.09 g, respectively), while the minimum was produced by parent P3(8) (19.62 g). It can be concluded that lint yield is the main component of productivity. The maximum significant ($P<0.01$) lint yield per plant (22.57 g) was produced by parent P1(1), followed by RA-2 (22.01 g), whereas the minimum seed cotton yield per plant (4.64 g) came from parental genotype P3(8) (**Table 4**). The trait ginning outturn differed significantly ($P<0.01$) among the parents (**Table 4**). The value ranged from 23.63 to 41.26% for the parents. Parent BC-119 produced the highest ginning outturn, while the lowest (23.63%) was produced by parent P3(8).

3.8 Earliness-Seed-Lint Index

The Bartlett's earliness index (Bartlett 1937) states that a higher value of the index indicates an earlier genotype. The parents exhibited non-significant variation, ranging from 0.58 to 1.11 for the earliness index (**Table 4**). Parent P1(1) had the highest value (1.11) for the earliness index, suggesting that this parent is classified as an early type, while P3(8) was considered late among all the parents, possessing the lowest value (0.58) of the earliness index. The ability to harvest cotton before

Table 4. Agronomic performance of 12 cotton genotypes for yield and related characters

Genotypes	PHT	NFB	NMB	NSB	SFB	DSQ	DFL	DBO	FBO	EBO
M1	67.71	5.22	1.11	11.94	2.89	57.03	70.33	139.03	163.11	178.67
RA-2	73.11	6.17	1.58	12.03	5.86	57.28	70.44	135.56	160.56	176.28
P1(1)	80.17	5.69	1.58	14.22	8.03	55.25	68.33	132.58	158.64	168.78
P3(8)	78.53	4.33	2.17	15.50	8.83	55.83	68.50	134.33	157.50	176.33
BC-119	87.41	5.11	2.31	14.78	10.64	55.97	68.97	131.25	156.47	166.97
BC-120A	73.66	5.94	1.50	13.44	6.69	56.75	73.08	133.72	164.33	176.50
BC-120B	85.66	6.17	1.56	13.17	6.94	58.14	71.08	137.22	173.53	195.00
CB-9	77.47	5.42	1.97	12.89	9.39	56.78	69.89	134.53	161.69	175.89
CB-12	77.48	6.00	1.75	14.22	8.97	57.25	70.25	137.64	162.44	174.50
CB-14	84.01	6.17	1.92	14.25	9.28	56.72	69.94	134.28	177.83	190.08
CB-15	80.28	6.36	1.33	13.28	6.42	57.72	70.72	137.00	171.39	182.56
CB-17	70.41	5.58	2.11	12.69	8.67	56.56	69.44	135.64	164.61	178.61
Mean	77.99	5.68	1.74	13.53	7.72	56.77	70.08	135.23	164.34	178.35
F value	ns	*	ns	**	ns	**	ns	**	**	**
CV%	10.16	11.8	27.06	6.36	34.69	1.12	2.28	1.68	2.22	2.35
LSD (0.05)	13.31	1.13	0.39	1.45	4.45	1.07	2.68	3.81	6.11	7.01

* Significance at 5% level, ** significance at 1% level of significance, ns= non-significant
PHT= plant height (cm), NFB= Node number bearing first fruiting branch, NMB= monopodial branches (no./plant), NSB= sympodial branches (no./plant), SFB= secondary fruiting branches (no./plant), DSQ= Days to square, DFL= first flowering (days), DBO= first boll opening (days), FBO= 50% boll opening (days), EBO= 80% boll opening (days)

Table 4. Continued

Genotype	NBP	PBR	PFP	SBW	SCY	LYP	GOT	EIX	SIX	LIX
M1	14.94	80.34	39.11	4.23	48.87	17.28	35.36	1.07	7.39	4.04
RA-2	15.94	74.26	56.51	4.44	68.47	22.01	32.13	1.02	7.99	3.78
P1(1)	22.36	79.63	56.18	4.51	68.09	22.57	33.14	1.11	7.23	3.58
P3(8)	12.67	56.40	10.92	4.32	19.62	4.64	23.63	0.58	9.47	2.93
BC-119	22.06	70.95	43.18	3.02	33.66	13.25	41.26	0.66	7.62	5.46
BC-120A	18.47	72.63	42.41	3.13	43.45	15.05	34.75	0.84	8.46	4.50
BC-120B	20.53	67.70	21.87	3.77	27.28	8.55	29.72	1.02	8.06	3.39
CB-9	17.75	68.04	37.30	4.37	42.94	14.03	33.00	0.83	9.91	4.89
CB-12	18.72	66.97	41.87	4.79	46.44	15.18	31.40	1.02	8.67	3.99
CB-14	21.58	69.33	33.14	4.43	53.11	17.52	32.82	0.78	9.39	4.59
CB-15	20.33	71.42	31.36	4.75	57.37	17.45	29.83	0.79	10.69	4.77
CB-17	18.72	69.28	37.03	4.47	39.92	13.88	34.76	0.85	10.05	5.35
Mean	18.67	70.58	37.57	4.19	45.77	15.12	32.65	0.88	8.74	4.27
F value	ns	*	**	**	**	**	**	ns	**	*
CV%	22.52	10.08	11.81	8.25	20.51	22.11	10.42	30.62	2.83	19.83

LSD (0.05)	7.12	12.02	7.66	0.57	15.75	5.75	5.78	0.43	0.43	1.42

* Significance at 5% level, ** significance at 1% level of significance, ns= non-significant
NBP= bolls per plant (no.), PBR= percent boll retention, PFP= first picking percentage, SBW= single boll weight (g), SCY= seed cotton yield (g/plant), LYP= lint yield (g/plant), GOT= ginning out turns (%), EIX= earliness index, SIX= seed index, LIX= lint index

it is damaged by unfavorable weather in some regions is crucial for enabling the crop to mature early. The parents varied significantly in terms of seed index (**Table 4**). The average value of the seed index ranged from 7.23 g to 10.69 g. CB-15 and CB-17 generated the maximum seed index (10.69 g and 10.05 g, respectively). The lowest seed index (7.23 g) was recorded by parent P1(1). Significant variation was noted among the parents for the lint index. The mean value ranged from 2.93 g to 5.46 g. Parents BC-119 and CB-17 yielded the highest lint index (5.46 g and 5.35 g, respectively). The lowest lint index (2.93 g) was produced by parent P3(8). Udaya et al (2023) also reported a similar lint index and found that the lint index ranged from 5.54 g to 8.35 g in cotton.

4 Conclusion

The percentage of molecular variance within the population and among the parental genotypes was 93% and 7%, respectively, as revealed by the AMOVA. The unweighted neighbor-joining tree, principal coordinate analysis, and heatmap clustered the parental genotypes into three major groups. In terms of agronomic performance, the parents BC-119 and P1(1) required the fewest days to achieve 80% boll opening, thereby being designated as early parents. Additionally, parents P1(1) and RA-2 recorded the highest seed cotton yield. As a result, these three parental genotypes could be selected as parents in the hybridization program to enhance cotton varieties with improved yield, earliness, and fiber quality.

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