

GENETIC INSIGHTS AND BREEDING IMPLICATIONS: ENHANCING COTTON YIELD AND QUALITY THROUGH BIPARENTAL SELECTION

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ABSTRACT

This study was performed in Egypt's Sakha Agriculture Research Station during the seasons 2015–2018 to study the efficiency of biparental progenies derived from inter-population random mating in F2 generation in a cotton cross. Intercrossing is allowed in cotton breeding populations. Although the best lines may include harmful alleles, thus early segregating generation recombination and hybridization are required. The F2 generation of the cotton cross (Giza70 TNB1), which obtained from Agriculture Research Center, Egypt, consisted of five male and eight female plants, was used to test biparental progeny from random interpopulation mating. About 28 and 24 families in BIPS1 and BIPS2 had their original parents were examined. All genotypes were assessed for agricultural and fiber qualities. The mean of families (BIPs2) was higher than BIPs1 for all measured metrics except lint yield, percentage, and uniformity ratio. BIPS2 families had more genotypic coefficients of variation (GCV) for features than BIPS1. Intercrossing affected correlation coefficients of BIPS1 and BIPS2 genotypes. Seed cotton yield and lint index genotypic correlations became considerable. Seed cotton yield estimated by seed index and first fruiting node location were altered from negative to positive. Among them, No.5, No.7, No.8, No.18, and No.19 had large yields and early ripening, as shown by their components. The top five families chosen for fiber length had gain values of 3.373% to 8.961% and 5.919 to 7.781% for fiber strength. The No.18 family exhibited good seed cotton production and fiber length. Intermating and self-pollination (BIPS2) may utilize non-additive gene effects and accumulate trait-influencing alleles. Continuing to select and evaluate families based on their large-scale reproductive performance will enhance the likelihood of producing genotypes with desired features. In conclusion, this study underscores the utility of continued selection based on discerned genetic parameters, genotypic correlations, and observed gains in facilitating the ongoing improvement of cotton breeding programs. By providing valuable insights into the intricacies of genetic variability and trait associations, this research holds implications not only for the advancement of cotton breeding practices but also for the broader agricultural landscape on both national and global scales.

Keywords: Biparental, selection efficiency, correlated response, hybridization, self-pollination

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INTRODUCTION

Identifying the genetic architecture of breeding material is essential to develop the most efficient breeding program, which led to rapid and maximum genetic enhancement in cotton (Abd El-Aty *et al.*, 2022). Cotton is one of the most important fiber commodities in the world. Egypt's exports and domestic textile

production rely primarily on the cotton as the most important fiber crop (Abo Sen *et al.*, 2022). The ultimate goal of a cotton breeding program is improving the yielding capacity and fiber properties of the commercially stable cotton varieties. In the preponderance of breeding programs, hybridization followed by pedigree selection is the standard procedure for developing new cotton varieties (El-Marzoky *et al.*,

2022). Several deleterious alleles are found to be fixed in elite lines, mandating frequent crossing. Moreover, interbreeding plays a major role when non-additive variance performs an important function in inheriting the trait of interest (El-Saadony *et al.*, 2022). Diminished of rg in populations with pairing phase linkages and grew in populations with repulsion phase linkages. Afterwards, it was substantiated by isolating homozygous lines equivalent to or superior compared to the initial F1 strain (Fouda *et al.*, 2022; Rajeev *et al.*, 2018). When compared to F3s, BIP had a higher heritability of yield and the component traits contributing to up yield (Gadallah *et al.*, 2022; Hassan *et al.*, 2021; Khedr *et al.*, 2022)). It was also discovered that the number of superior offspring produced by inbreeding was higher than that produced by the F4 generation for the majority of characteristics (Saad *et al.*, 2022; Sehsah *et al.*, 2022; Selim *et al.*, 2022). Biparental progenies and F3 progenies had higher means for most of the characteristics as compared for their overall mean values. This might be because biparental progenies had larger heterozygosity than F3 progenies (Azeem *et al.*, 2023; Ul-Allah *et al.*, 2023). It was revealed that F2 biparental offspring originating from F3 families are unable to break the negative link between several features in two Egyptian cotton crosses. Moreover, the mean values for preferred direction rose in biparental progeny compared to F3 families for all characteristics except seed index and micronaire reading (Aripov and Ioelovich 2020). This was the case for all attributes except seed index and micronaire reading (AL-Hibbiny 2015). In comparison to the next generations, the phenotypic coefficients of variation PCV and GCV for fiber cotton yield/plant, bolls/plant, and boll weight were found to be higher in the F2 generation. This suggests that the amount of genetic variety that remained in these materials was adequate to provide a significant amount of improvement via selecting for better offspring. Estimates of both the heritability and the PCV and GCV followed the same trend. When compared to F2, the use of intermating results in several formerly negative correlations between attributes being transformed into positive ones. These relationships include seed cotton yield and earliness index, lint index, and fiber length.

The Giza70 TNB1 cotton variety was developed at the Giza Cotton Research Institute in Egypt. It is a cross between two popular varieties i.e., Giza86 and Giza92. This hybridization has resulted in a plant that combines the best traits of both parent varieties, making it a highly desirable option for cotton growers. The fibers produced by the Giza70 TNB1 cotton variety are long, strong, and fine, making them ideal for high-quality textile production. The fibers also exhibit a high degree of uniformity, ensuring consistent quality in the finished product. Giza70 TNB1 has a higher yield potential compared to other cotton varieties, making it a profitable choice for farmers. Due to the lack of literature on this

crossed line so we conducted this experiment to present a sufficient literature on these crosses which can use in further breeding programs. This investigation aimed mainly to evaluate the viability of biparental offspring in the F2 generation of a cotton hybrid resulting from random mating between members of different populations.

MATERIALS AND METHODS

The current study was carried out in the Sakha Agriculture Research Station, Kafrelsheikh, Agri-cultural Research Center, Egypt, from the seasons of 2015 to 2018, respectively. The F2 population of the cotton cross (Giza 70 (G.70) TNB1) was the primary source material was used in this experiment. The progenitors had excellent qualities in terms of fiber quality, yield, and earliness, and there was a wide variety of geographic origins among them. In this study, G.70 (Giza 59Ax Giza 51B) is classified as an extra-long staple and TNB1 (Indian) is classified as an extra-long staple with a high particle percentage and increased earliness, the difference between the two types of tobacco is that G.70 (Giza 59Ax Giza 51B) is an extra-long staple.

Breeding procedures: During the growing season of 2015, F2 seeds derived from the cross Giza70 x TNB1 were germinated and planted. The North Carolina Design1 was used to carry out hybridization between five male and eight female of F2 plants that were chosen at random (Comstock and Robinson 1948, 1952). During the 2016 growing season, forty hybrids were selfed and assessed between individual plants. In addition to being compared to their original progenitors using a randomized full block design with three biological replicates. These comparisons were made using a methodology known as selfing. The duplicate was planted in a single row that was five meters long. There was a distance of 70 cm between rows, and 30 cm separated the slopes. And each plant was capable of self-pollination. Within each family, selection was carried out based on the quantity of retained bolls as well as the individual plant's level of productivity in comparison to that of its original progenitors. Independent collections of both selfed and openly pollinated bolls from each plant were made. Twenty-eight biparental self-pollinated (BIPs1) plants were created as a result of the selection of inter and intra hybrids at the end of the season. These plants were intended to be sown the following year. The rationale behind choosing specific plants for hybridization was based on their performance in these key traits and their potential to contribute to the desirable characteristics to the breeding population. In the course of the 2017 growing season, a total of 28 biparental self-pollinated (BIPs1) were examined. In addition to their self-pollinated and self-parented parents. At the end of

the growing season, a total of 24 seedlings were generated as a result of hybridization both across and within families. The selection of biparental self-pollinated (BIPs2) is done based on the yield and its related components. The key findings from the breeding cycles BIPS1 and BIPS2, highlighting the progression of the study over time (Table 1). In BIPS1, initial selection

and hybridization led to the creation of biparental self-pollinated (BIPs1) plants, which were further evaluated and refined in subsequent cycles. The results from BIPS2 reflected the outcomes of additional rounds of self-pollination and analysis, providing insights into the performance and traits of the progeny compared to their original progenitors.

Table 1. Procedure and timetable of BIPS1 and BIPS2.

2015	F ₂ plants were crossed using North Carolina Design-1
2016	40 hybrids were planted and self-pollinated for the plants
2017	28 superior plants and selection for the plants as Families (BIPs ₁) were evaluated with original parents and self-pollinated
2018	24 superior plants and the (BIPs ₂) were evaluated with original parents

The Giza70 TNB1 cotton variety, developed at the Giza Cotton Research Institute in Egypt, is a cross between Giza86 and Giza92. This hybridization has resulted in a plant that combines the best traits of both parent varieties, making it a highly desirable option for cotton growers. The fibers produced by the Giza70 TNB1 cotton variety are long, strong, and fine, making them ideal for high-quality textile production. The fibers also exhibited a high degree of uniformity, ensuring consistent quality in the finished product. Giza70 TNB1 has a higher yield potential compared to other cotton varieties, making it a profitable choice for farmers. Additionally, the plants are known for their disease resistance, protecting them from common pests and pathogens. The cotton cross (Giza70 TNB1) is also known for its environmental benefits. These plants efficiently use water and nutrients, making them a sustainable choice for cotton production. Moreover, the high-quality fibers produced by this variety can reduce the environmental impact of textile production by ensuring a longer lifespan for textiles made from them. In conclusion, the Giza70 TNB1 cotton variety offers a range of benefits to both farmers and consumers due to its superior fiber quality, high yield potential, and environmental sustainability. This variety is likely to play an important role in meeting the growing demand for high-quality cotton in a sustainable and environmentally friendly manner. The studied traits included the position of first fruiting node (FFN), number of days to first flowering (DFF), boll maturation period (BMP), earliness index (EI%), boll weight (g) (BW), seed cotton yield (g/plant) (SCY/P), lint cotton yield (g)/plant (LCY/P), lint percentage (L%), seed index (g) (SI), lint index (g) (LI), fiber length at 2.5% span length (2.5%SL), uniformity ratio (UR), fiber strength (g/tex) (FS) and micronaire reading (MR).

Statistical analysis: Data were subjected to statistical analysis of variance (Comstock and Robinson 1952) and developed according to (Kearsey, Pooni, and 1996; Singh and Pawar 2002). The mean, range, genotypic and phenotypic coefficients of variation were estimated for all the studied traits in both biparental selection cycles BIPS₁

and BIPS₂. Heritability was calculated (Kearsey, Pooni, and 1996). Genotypic correlation coefficients along the studied characters in BIPs₁ and BIPs₂ were estimated (Falconer and Muckey 1996).

Observed direct gain for (BIPS₂) families was estimated by the following formula given by Steel *et al.*, 1996).

$$\text{Observed directly gain} = \frac{(\bar{X}_f - \bar{X}_{bp})}{\bar{X}_{bp}} \times 100$$

Where: \bar{X}_f = denotes the mean of each family

\bar{X}_{bp} =denotes better parent mean

This formula quantifies the percentage increase or decrease in the mean performance of the trait of interest in the offspring generation (BIPS₂) relative to the mean performance of the better parent. A positive value indicated an improvement in the trait compared to the better parent, while a negative value suggested a decline. The magnitude of the observed direct gain provides insights into the effectiveness of the breeding program in enhancing the desired traits across successive generations.

Cluster analysis: Soft clustering was performed in R software (R-Core-Team 2021) using the Fuzzy C-means algorithm using the Squared Euclidian distance dissimilarity measure of the studied traits (Bezdek 1981). Squared Euclidian distance was computed after the standardization of the data as the studied traits were measured with different units. Standardization is the subtracting of each value from the mean and then it was dividing the result by standard deviation. Because LCY and EI were the highest traits in variance, they were picked up to construct the distance matrix applying the Squared Euclidian coefficient and then visualize clustering results among all the genotypes. To define the optimum number of clusters, 42 clusters validity indices were used (Charrad *et al.*, 2014; Desgraupes and Desgraupes, 2018).

Heatmap was drawn using (*pheatmap*) package in R software (R-Core-Team 2021). The heatmap shows the relationship between the genotypes and the

standardized traits based on the color scale. Red cells in the heatmap are representing high values of the traits, while blue cells are representing low values of the traits. The data have different measuring units. Thus the data were standardized by subtracting the mean from each value and dividing the result by the standard deviation.

RESULTS

Genetic parameters: The majority of the characteristics investigated in both biparental selection cycles (BIPS1 and BIPS2) showed a slight increase in a desirable direction in the BIPS2 population (Table 2). This performance may be due to the recombination of desirable alleles in BIPs2 and the dissolution of some of the relationships between the variables under consideration.

Estimates of PCV, GCV, and heritability facilitated the selection of breeders. Due to the disruption of the coupling and repulsion phase connection in BIPs1, GCVs for the location of the first fruiting node, days number to first flowering, boll maturation duration, seed index, and lint index were greater for BIPS2 families than those in BIPs1 families. Qin and Horvath (2020) results are in harmony with our results. BIPs progeny had a greater coefficient of variation than F3 progeny because interbreeding in the F2 population disrupted the unfavorable strong negative connections between yield and fiber characteristics in F3 families.

Boll maturation period (BMP), earliness index (EI%), boll weight (g) (BW), seed cotton yield (g)/plant (SCY/P), lint cotton yield (g)/plant (LCY/P), lint percentage (L%), seed index (g) (SI), lint index (g) (LI), fiber length at 2.5% span length (2.5%SL), uniformity ratio (UR), fiber strength (g/tex) (FS) and micronaire reading (MR). Estimates of the coefficient of variation for earliness index, seed cotton yield, lint cotton yield, seed index, and lint index characteristics revealed variations in GCV and PCV. It could be attributable to the magnitude of non-additive and adverse environmental impacts. Large differences between GCV and PCV suggested that the environment affects the expression of these traits (Gonzales, Cavero, and Soto 2021). Consequently, careful selection might be used to improve the performance of families (Mahmood *et al.*, 2020). It was discovered that the PCV and GCV were generally larger in magnitude for all studied traits in the F2 generation compared to advanced generations F3 and F4. This indicates that the magnitude of the genetic variability persisting in this material was adequate for providing a substantial amount of improvement through the selection of superior offspring. Concurrently, the PCV was higher than the GCV for all characteristics that were evaluated. In the vast majority of instances, the significant disparity between the PCV and the GCV was

found across three generations, showing that powerful genetic influences are at play.

The BIPS2 family exhibited higher estimates of heritability for all variables, except for boll weight and fiber attributes, when comparing broad-sense heritability to the BIPS1 family. Additionally, the heritability values in the broad sense of biparental self-pollinated1 (BIPS1) and biparental self-pollinated 2 (BIPS2) were larger than fifty percent (50%) for all of the investigated characteristics. These results were in harmony with those obtained by (Gaballah *et al.*, 2022; Sorour *et al.*, 2013). In addition, the genetic variation has not been fully exhausted and that there is the possibility for the ongoing growth of the qualities that were investigated by using the same selection strategy to consecutive generations of organisms. This may be done by selecting individuals from the same population using the same criteria each generation. The level of genetic variety shown by (BIPS2) families was much higher than those in BIPS1 families. Therefore, heritability in its broadest sense, is insufficient to offer a clear understanding of the nature of inherited characteristics on its own. Heritability and genetic variability presented a clear picture of genetic advancement when they were combined. In the case of BIP, heritability was higher in respect of yield and its component characters than in F3s (Zhong *et al.*, 2021). Also it was revealed that estimates of heritability for the majority of traits increased considerably when biparental families were included (Suvi *et al.*, 2021; Yehia and El-Hashash 2019). Differences in significance between the BIPs1 and BIPs2 families and the better parent in the desired direction (Tables 2 and 3). Indicating that it is possible to pick superior families for the qualities that were researched and that are regarded to be useful indicators for the cotton breeder. Some families had superior behavior for a greater number of qualities, and they might be considered for nomination of additional breeding selection to increase these characteristics.

Genotypic correlation: The investigation of genotypic correlation is crucial for breeding programs. Correlation between It is possible that key attributes will make the interpretation of the findings easier and will offer a basis for the construction of more successful breeding plans that combine several characteristics that are desired. The genotypic correlation values for biparental (BIPS1) and (BIPS2) markers (Table 4). When moving from BIPS1 to BIPS2, several interactions between features exhibited shifts in both value and direction. In BIPS2, the correlation value between seed cotton yield and lint index went from being non-significant to being considerably positive (-0.151 to 0.445). In BIPS1, same movement occurred from non-significant to significant. In addition, the value of the association between seed cotton yield and seed index flipped from having a negative value (-0.228) to having a positive value (0.366). In addition, these

genetic connections between earliness index, seed cotton yield, and lint yield have a positive and highly significant correlation value ranging from (0.995** to 0.998**) and (0.978** to 0.970**), respectively. These correlation values are indicative of the progression of genetic advancement. These changes in value and direction could be a result of linkage breakdown. (Miller *et al.*, 1958) also observed a rise in the amount of genotypic correlation in populations displaying repulsion phase linkages in cotton. While simultaneously reporting a drop in the magnitude of genotypic correlation in populations exhibiting coupling phase linkages in cotton. Also, (Qiu *et al.*, 2020) intercrossing and recurrent selection are two methods that have been proposed for use in the early segregating generations of cereals that are capable of self-pollination, such as wheat. These methods are intended to break negative links and maintain higher diversity for successive selections (Punia, Sangwan, and Nimbal 2017). It was shown that interbreeding within the F2 generation successfully broke unwanted connections. Therefore, it is feasible that a rearrangement of the genes responsible for correlation among some qualities resulted in the production of unique combinations. These novel combinations likely resulted from transitions from a coupling phase to a repulsion phase. Thus, a reorganization of the genes responsible for correlation among some characteristics led to the formation of novel combinations. Similarly, (Farg 2017) found that the relevance of the genetic correlation between the economic qualities in cotton, as well as the negative correlations between yield and fiber attributes, could be converted to a positive correlation via biparental mating. Moreover this modification would not affect how crucial the genetic correlation between the economic features. (Areej *et al.*, 2021) observed that genotypic correlation (r_g) followed the same pattern as phenotypic correlation (r_p), but in the majority of instances, r_g held a bigger value than r_p , which suggests that genetic factors were more prominent in the manifestation of those features than the effects of the environment (Farg 2022). Positive, extremely significant and substantial genotypic correlation values were discovered between seed cotton yield and lint yield and lint percentage, as well as seed index and lint index, respectively. The values of these correlations were 0.514*, 0.468*, 0.718**, and 0.813**, correspondingly. Only a highly phenotypic correlation value was found between seed cotton yield and lint yield (0.981**), but it was not statistically significant. The acceptable amount of genetic diversity lends support to the link between the numerous yields contributing factors.

Observed gain: The observed increase in yield and its component features for the majority of families was selected based on seed cotton yield in BIPS2, compared to the superior progenitor (Table S1). These families

were chosen because they had higher yields than the superior progenitor. The observed gain values for the top five families in terms of seed cotton yield varied from 35.511% to 55.193%, and the earliness index ranged from 20.283% to 23.951%. These findings revealed that the genotypes had a high seed cotton output, which was characterized by more earliness in BIPS2 than in TNB1 (the superior progenitor). Additionally, the earliness of BIPS2 was larger than that of (TNB1). In terms of seed cotton output, the high mean values of the self-pollinated earliness index (BIPS2) seemed to be boosted. The top five families picked had an earliness index that ranged anywhere from 55.193% to 36.223%, while their seed cotton yield varied anywhere from 55.193% to 36.223%. According to these findings, several families, such as No. 5, No. 7, No. 8, No. 18, and No. 19, had high yield and larger degree of earliness than the other parameters. It's possible that this is because to the genetic association that exists between early maturity and seed cotton output (0.998%). Therefore, the selection for such families, which are defined by a high yield and a larger degree of early maturity in following generations, will be effective. The observed gain values of the top five families selected based on fiber length varied from 3.373% to 8.961% for fiber length, and from 5.919% to 7.788% for fiber strength. In the meanwhile, these values were observed. In addition, family No. 18 had a very considerable rise in both the length of their fibers and the quantity of seed cotton they produced. Through the use of intermating and then in (BIPS2), it is possible to credit the exploitation of non-additive gene effects to the accumulation of beneficial alleles that influence attributes (El-Mowafi *et al.*, 2021). In conclusion, the earlier findings shown that there were certain families that displayed greater superior qualities in addition to a high yield as well as a good fiber quality. These families were distinguished by their high yield and excellent fiber quality. Therefore, continuing selection and assessment of these BIPS families based on their performance might potentially increase the possibility of obtaining genotypes with desired traits.

It's observed that all the genotypes were grouped into 2 clusters (Figure 2). There is an overlapped region between the two clusters where any genotype that falls in that region is belonging to the 2 clusters with a membership degree. Each genotype has a membership value ranging from 0 to 1 (Table 5). These values explain the degree of belonging of a certain genotype to a certain cluster. When a membership value is close to 1, high belonging is observed, on the other hand, a membership value close to 0 reveals lower belonging. The membership of all genotypes to their clusters was distinct, over 0.80, and 0.90, except for a few genotypes such as G2, G4, and G20, which have memberships of 0.71 and 0.68 to cluster 2, and 0.71 to cluster 1. These genotypes exist in the overlapping region and far from the center of the genotypes (Figure 1).

Table 2. Mean performance for all studied traits of biparental self-pollinated 1 (BIPSI) progeny.

Genotype	BMP	EI %	BW(g)	SCY/P(g)	LCY/P(g)	L%	SI(g)	LI(g)	2.5%SL	UR	FS (g/tex)	MR
1	53.7**	68.81	3.49	85.42**	33.18**	38.83**	10.80	6.86**	35.65**	89.60**	41.30	4.41
2	56.00**	60.88	3.46	66.52	24.90	37.43**	10.80	6.46**	36.52**	89.35**	41.43	4.39
3	52.00**	68.85	3.67*	87.49**	32.24**	36.84**	11.03	6.43**	35.48**	89.97**	43.37**	4.36
4	54.53**	65.29	3.53	79.75*	29.71**	37.06**	10.75	6.35*	36.56**	89.39**	44.73**	4.12**
5	55.47**	67.75	4.00**	86.89**	32.99**	38.00**	11.72**	7.18**	36.73**	87.12	43.70**	4.34
6	54.00**	62.08	3.58	70.82	27.40	38.74**	12.23**	7.73**	36.14**	88.41**	42.77**	4.21**
7	55.47**	68.91	3.43	80.89*	30.37**	37.49**	11.63**	6.98**	36.03**	88.63**	42.47**	4.04**
8	54.20**	69.56*	3.57	88.22**	32.67**	37.06**	11.92**	7.02**	35.91**	89.17**	38.80	4.23**
9	55.33**	68.91	3.71*	90.73**	34.92**	38.47**	11.56**	7.23**	34.39*	87.79	42.63**	4.53
10	54.40**	65.94	4.00**	78.71*	30.71**	39.05**	11.21*	7.18**	35.35**	89.13**	40.23	4.06**
11	54.93**	65.03	3.60	75.59	29.41*	38.88**	11.75**	7.47**	35.59**	88.32**	41.63	4.10**
12	54.67**	69.47*	3.63	86.92**	34.24**	39.40**	11.33*	7.37**	35.19**	87.63	39.63	4.27*
13	54.53**	58.43	3.37	61.59	23.34	37.88*	11.00	6.72**	37.40**	86.88	40.50	3.97**
14	55.47**	61.38	3.62	71.20	27.44	38.55**	11.08	7.57**	37.40**	87.28	41.03	4.56
15	54.00**	65.25	3.50	80.27*	31.94**	39.72**	11.77**	7.77**	35.27**	89.97**	40.90	3.73**
16	55.47**	63.15	3.63	74.32	28.71*	38.60**	11.76**	7.39**	36.37**	88.72**	42.57**	4.33
17	54.20**	71.66**	3.57	102.28**	38.49**	37.72**	11.85**	7.19**	37.20**	89.46**	40.70	4.43
18	55.33**	62.76	4.00**	70.08	27.29	38.82**	11.04	7.04**	37.67**	89.08**	44.70**	4.60
19	54.40**	59.94	3.70*	68.94	25.66	37.25**	12.83**	7.63**	37.80**	88.63**	41.73	4.80
20	54.93**	60.03	3.43	65.82	25.41	38.35**	11.43**	7.12**	35.53**	89.27**	41.97*	4.47
21	54.67**	66.10	3.53	76.71	28.90*	37.72**	11.15	6.75**	35.73**	89.09**	38.83	4.00**
22	54.53**	68.49	3.49	87.92**	32.87**	37.53**	11.41**	6.86**	36.10**	88.71*	39.67	4.50
23	55.47**	62.49	3.45	70.11	27.00	38.41**	11.35*	7.08**	35.50**	89.48**	40.73	4.57
24	54.00**	63.95	3.71*	77.78	30.18**	38.82**	11.56**	7.34**	35.90**	88.85**	40.17	4.33
25	55.47**	64.98	3.69*	78.79*	31.05**	39.48**	11.79**	7.70**	35.10**	89.27**	40.03	4.37
26	54.20**	63.47	3.42	73.71	28.90*	38.94**	11.35*	7.24**	35.50**	89.09**	44.57**	4.40
27	55.33**	75.41**	3.57	109.70**	41.73**	38.06**	10.71	6.59**	37.93**	88.71**	41.30	4.37
28	55.27**	77.04**	3.33	110.39**	43.96**	39.83**	11.05	7.32**	35.77**	89.48**	43.63**	4.23**
TNB ₁	66.27	63.10	3.43	60.43	21.11	34.93	10.53	5.66	33.80	87.43	41.24	4.43
Giza 70	64.40	60.95	3.37	58.43	20.99	35.92	9.73	5.46	34.97	88.57	40.00	4.35
LSD0.05	1.392	6.171	0.232	16.935	6.616	1.275	0.623	0.534	0.289	0.654	0.662	0.149
LSD0.01	1.874	8.308	0.313	22.800	8.908	1.717	0.839	0.719	0.391	0.880	0.891	0.188

Both **, and **** indicated significant at 0.05 and 0.01 levels of probability, respectively, compared with better parents, boll maturation period (BMP), earliness index (EI%), boll weight (g) (BW), seed cotton yield (g/plant) (SCY/P), lint cotton yield (g/plant) (LCY/P), lint percentage (L%), seed index (g) (SI), lint index (g) (LI), fiber length at 2.5% span length (2.5%SL), uniformity ratio (UR), fiber strength (g/tex) (FS), and micronaire reading (MR).

Table 3. Mean performance for all studied traits of biparental self-pollinated 2 (BIPS2) families.

Genotype	BMP	EI %	BW(g)	SCY/P(g)	LCY/P(g)	L %	SI(g)	LI(g)	2.5%SL (mm)	UR	FS (g/tex)	MR
1	49.73**	68.35**	3.69**	75.64	26.38	37.29**	11.59**	6.90**	35.47	86.70	43.60**	4.03**
2	49.20**	71.47**	3.73**	78.28*	26.71	36.46**	11.95**	6.87**	35.77**	86.50	43.87**	4.33**
3	50.80**	68.66**	4.00**	76.03	27.84*	39.18**	11.68**	7.54**	35.10	86.60	42.93**	4.40
4	48.27**	70.67**	3.40	80.56**	27.80*	36.79**	12.32**	7.17**	35.80**	87.10**	43.60**	4.47
5	49.13**	73.57**	3.80**	86.00**	30.01**	37.06**	12.00**	7.07**	35.50	87.50**	43.73**	4.30**
6	50.73**	69.56**	3.53	79.68**	27.79*	37.23**	12.49**	7.41**	35.23	86.43	43.20**	4.17**
7	49.93**	75.82**	3.63	98.50**	35.32*	37.85**	11.96**	7.28**	35.67**	86.77	43.60**	4.33**
8	51.80**	74.36**	3.77**	92.38**	33.09**	37.75**	12.37**	7.51**	37.17**	87.60**	45.60**	4.00**
9	50.20**	71.31**	3.75**	84.71**	30.21**	37.86**	12.12**	7.40**	36.77**	86.50	45.33**	4.60
10	49.20**	71.81**	3.71**	83.69**	30.33**	38.57**	11.97**	7.52**	37.50**	87.27**	46.00**	4.00**
11	51.73**	71.31**	3.85**	82.64**	29.83**	38.42**	12.23**	7.63**	37.30**	86.43	44.67**	4.47
12	48.20**	71.67**	3.73**	83.03**	30.21**	38.71**	11.80**	7.45**	37.50**	87.40**	44.40**	4.10**
13	48.07**	68.52**	3.69**	72.65	23.94	35.38	12.15**	6.65**	35.93**	86.47	44.93**	4.60
14	50.87**	69.72**	3.70**	75.95	26.76	37.69**	12.70**	7.69**	37.63**	87.67**	44.67**	4.01**
15	50.80**	68.57**	3.63*	68.06	23.91	37.90**	11.85**	7.24**	36.97**	87.33**	45.73**	4.67
16	48.27**	66.42	3.72**	71.37	24.92	37.53**	12.33**	7.41**	37.17**	86.83*	44.53**	4.53
17	50.13**	67.95*	3.79**	74.44	26.50	38.16**	12.31**	7.60**	36.57**	86.80*	46.13**	4.67
18	49.13**	72.98**	3.81**	86.46**	30.53**	37.49**	12.47**	7.48**	38.10**	87.60**	44.13**	4.67
19	49.13**	74.47**	3.63	91.97**	32.53**	37.36**	12.28**	7.34**	35.80**	87.53**	44.27**	4.63
20	50.87**	72.10**	3.81**	80.82**	28.47**	37.55**	12.57**	7.56**	37.13**	86.33	44.00**	4.57
21	51.87**	68.87**	3.65	74.82	26.53	38.03**	10.27	6.31**	36.57**	86.27	43.87**	4.23**
22	50.13**	67.55*	3.99**	75.40	26.17	37.17**	12.40**	7.34**	36.73**	88.40**	44.13**	4.23**
23	51.93**	67.16*	3.85*	70.65	23.78	36.25*	11.32*	6.45**	36.27**	87.33**	45.20**	4.27**
24	50.13**	70.07**	3.71**	72.67	25.97	38.38**	10.47	6.52**	37.03**	87.13**	45.07**	4.37*
TNB ₁	67.33	63.50	3.40	69.27	24.15	34.87	10.33	5.53	34.00	85.43	41.47	4.47
Giza 70	65.50	61.17	3.33	60.47	21.38	35.37	10.00	5.76	35.17	86.57	40.80	4.33
LSD.0.05	0.436	3.313	0.202	7.301	2.920	1.072	0.673	0.510	0.344	0.249	0.551	0.087
LSD.0.01	0.593	4.503	0.274	9.926	3.979	1.460	0.915	0.693	0.466	0.339	0.750	0.119

Both ***, and ***, indicated significant at 0.05 and 0.01 levels of probability, respectively, compared with better parents. Boll maturation period (BMP), earliness index (EI%), boll weight (g) (BW), seed cotton yield (g/plant) (SCY/P), lint cotton yield (g/plant) (LCY/P), lint percentage (L%), seed index (g) (SI), lint index (g) (LI), fiber length at 2.5% span length (2.5%SL), uniformity ratio (UR), fiber strength (g/tex) (FS), and micronaire reading (MR).

Table 4. Genotypic correlations between all traits of biparental selfed pollinated 1(BIP_{S1}) and biparental selfed pollinated 2(BIP_{S2}) families.

Traits	families	FFN	DFE	BMP	EI%	BW (g)	SCY/P (g)	LCY/P (g)	L %	SI (g)	LJ (g)	2.5%SL (mm)	UR	FS (g/tex)
BMP	BIPS1	0.480**	0.675**											
	BIPS2	-0.051	0.262											
EI%	BIPS1	-0.091	-0.110	-0.146										
	BIPS2	0.236	-0.453*	-0.101										
BW	BIPS1	-0.403*	-0.235	0.019	-0.042									
(g)	BIPS2	-0.390*	0.153	0.330	-0.191									
SCY/	BIPS1	-0.105	-0.174	-0.085	0.995**	-0.115								
p(g)	BIPS2	0.148	-0.415*	-0.090	0.998**	-0.121								
LCY	BIPS1	-0.151	-0.120	-0.034	0.978**	-0.111	0.991**							
/p(g)	BIPS2	0.137	-0.446*	-0.023	0.970**	-0.058	0.980**							
L%	BIPS1	-0.407*	0.376*	0.349	-0.017	0.097	0.038	0.169						
	BIPS2	0.038	-0.282	0.299	0.177	0.257	0.204	0.393*						
SI	BIPS1	-0.081	0.264	-0.067	-0.307	0.196	-0.225	-0.238	-0.074					
(g)	BIPS2	-0.362	-0.246	-0.249	0.226	0.033	0.335	0.266	-0.241					
LI	BIPS1	-0.341	0.470**	0.197	-0.254	0.219	-0.151	-0.067	0.661**	0.699**				
(g)	BIPS2	-0.323	-0.402*	-0.032	0.323	0.207	0.445*	0.500**	0.411*	0.786**				
2.5%SL	BIPS1	0.242	-0.100	0.233	-0.165	0.104	-0.065	-0.101	-0.254	-0.057	-0.226			
(mm)	BIPS2	-0.505**	-0.412*	0.074	0.020	0.190	-0.042	0.040	0.370	0.129	0.356	-0.328		
UR	BIPS1	0.276	-0.225	-0.459*	0.282	-0.255	0.238	0.218	-0.185	-0.144	-0.235	0.356		
	BIPS2	-0.249	-0.228	-0.115	0.118	0.248	0.146	0.129	-0.033	0.232	0.200	0.138	0.041	
FS (g/tex)	BIPS1	-0.145	-0.126	0.059	-0.023	0.123	0.015	0.024	-0.031	-0.157	-0.128	0.636**	0.245	
	BIPS2	-0.549**	-0.039	0.131	-0.125	0.082	-0.214	-0.177	0.084	0.037	0.100	0.298	-0.055	0.220
MR	BIPS1	-0.188	-0.196	0.175	-0.155	0.274	-0.036	-0.047	-0.054	0.139	0.056	0.376	0.055	0.182
	BIPS2	-0.100	-0.214	0.040	-0.066	0.119	-0.089	-0.066	0.085	0.015	0.065	0.376	0.055	0.182

Both ** and *** indicated significant at 0.05 and 0.01 levels of probability, respectively, compared with better parents.

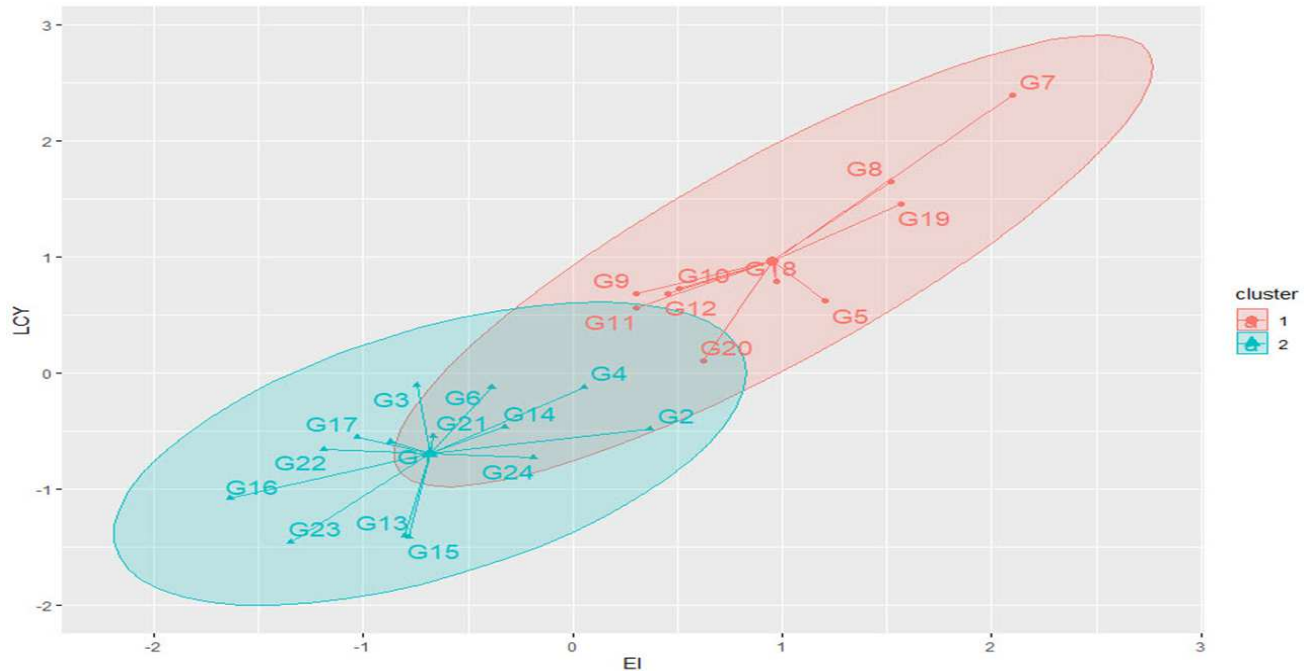


Figure 1. Results of soft clustering using Fuzzy C-means algorithm.

Table 5. Average of the boll maturation period (BMP), earliness index (EI%), boll weight (g) (BW), seed cotton yield (g)/plant (SCY/P), lint cotton yield (g)/plant (LCY/P), lint percentage (L%), seed index (g) (SI), lint index (g) (LI), fiber length at 2.5% span length (2.5%SL), uniformity ratio (UR), fiber strength (g/tex) (FS) and micronaire reading (MR) for the two clusters.

	BMP	EI	BW	SCY	LCY	LP	SI	LI	SL	UR	FS	MR
Cluster1	49.9	72.9	3.75	87.0	31.1	37.9	12.2	7.42	36.8	87.1	44.6	4.37
Cluster2	50.1	68.8	3.72	74.7	26.1	37.4	11.8	7.08	36.3	87.0	44.4	4.36

Table (6) showed the averages of the studied traits for the two clusters. The traits that differentiate easily between the two clusters were LCY, SCY, and EI, where their values in cluster 1 were (72.9, 87.0, and 31.1)

which surpassed cluster 2 (68.8, 74.7, and 26.1). Based on these results, the selection from cluster 1 is better than the selection from cluster 2 in breeding programs.

Table 6. Genotypes' membership.

Genotype	Cluster 1	Cluster 2	Genotype	Cluster 1	Cluster 2
G1	0.006	0.994	G13	0.061	0.939
G2	0.285	0.715	G14	0.052	0.948
G3	0.105	0.895	G15	0.062	0.938
G4	0.316	0.684	G16	0.077	0.923
G5	0.966	0.034	G17	0.018	0.982
G6	0.152	0.848	G18	0.995	0.005
G7	0.833	0.168	G19	0.939	0.062
G8	0.925	0.075	G20	0.717	0.283
G9	0.887	0.114	G21	0.007	0.993
G10	0.947	0.053	G22	0.026	0.974
G11	0.847	0.153	G23	0.082	0.918
G12	0.927	0.074	G24	0.053	0.947

The relationship between the standardized traits and the genotypes based on the color scale (Figure 2). The genotypes (G7) have higher FFN, LCY, SCY, and EI

(red color). The higher LCY and SCY were due to the higher value of EI, meaning that the main contribution to LCY and SCY was EI. Less contribution to LCY and

SCY was observed for FFN as its color was lighter red. However, regarding the genotype (G15) which has the lowest LCY and SCY. Its lowest LCY and SCY in the

genotype (G15) were mainly due to the lowest BW which has the main effect on its LCY and SCY.

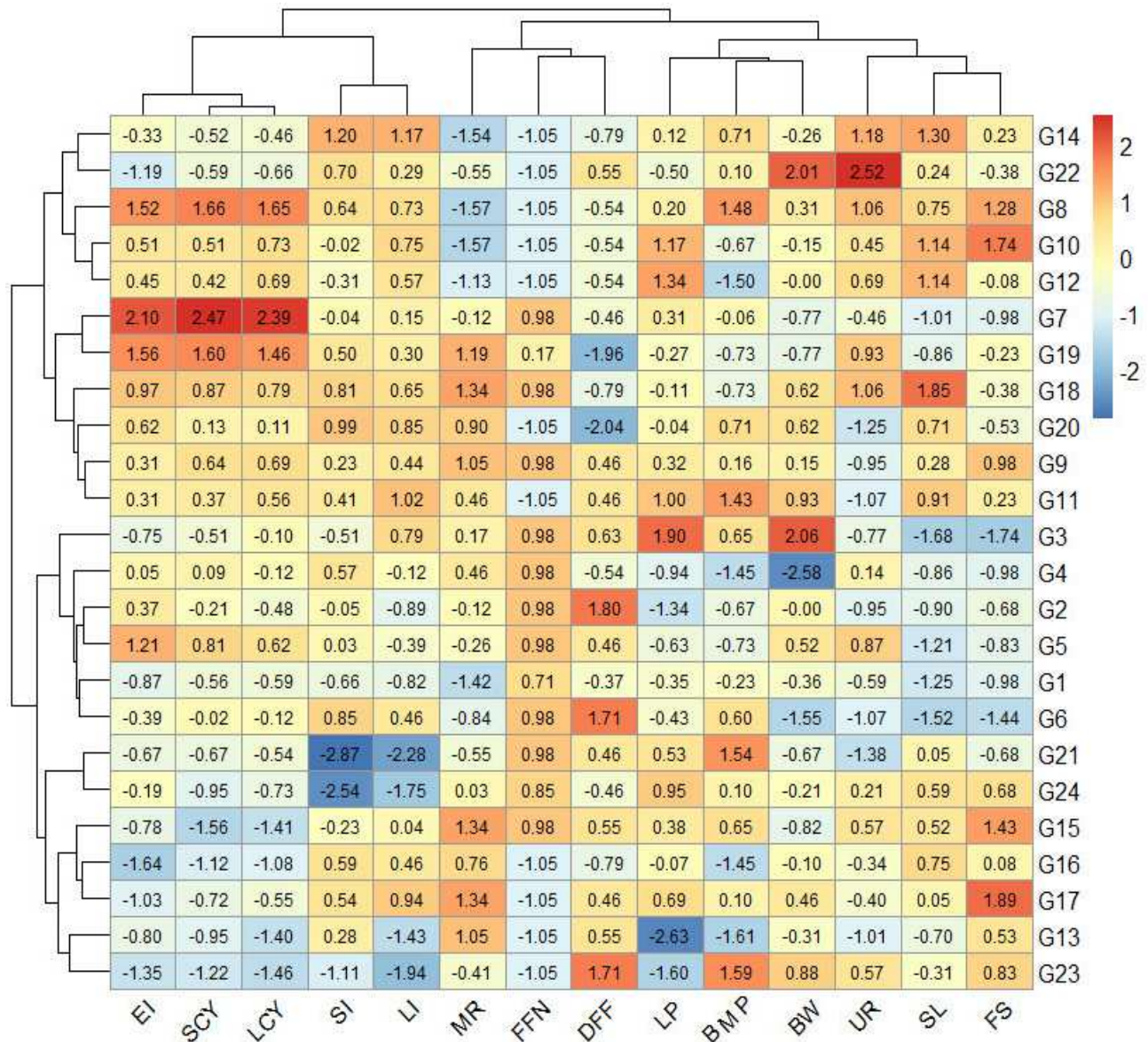


Figure 2. Heatmap of the relationship between the standardized traits and genotypes.

The dissimilarity among the genotypes based on all the standardized data using a color scale where higher values of similarity or lower values of dissimilarity are associated with darker blue color and the opposite is true (Figure 3). The genotypes (G7, G8, and G19) were more dissimilar to the other genotypes than the other genotypes. These genotypes were in the same cluster

(cluster1) (Figure 1). These genotypes can be represented by one of them in the breeding program.

Figure (4) illustrates the density of the studied traits. All the studied traits have one peak except for FFN, DFF, SL, and UR. The number of peaks indicates the homogeneity of that trait; a one-peak trait is more homogenous than a trait with two peaks.

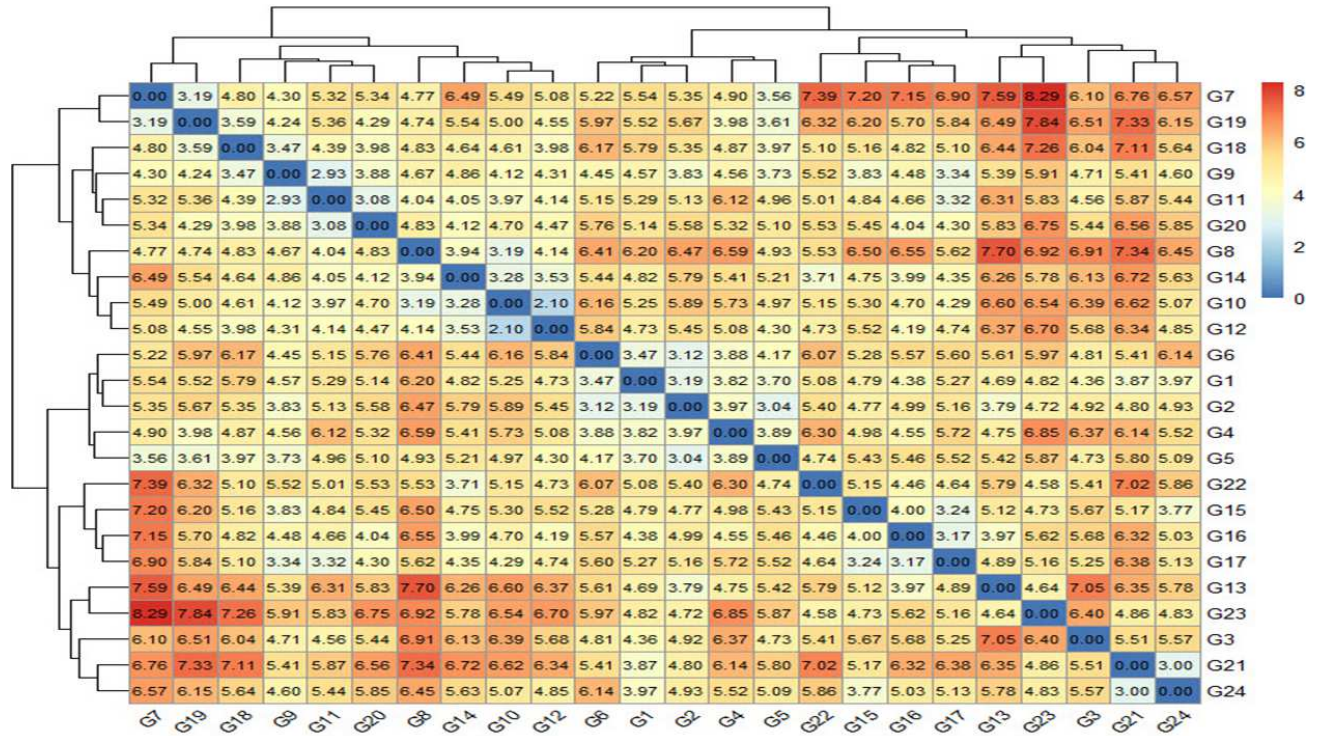


Figure 3. Heatmap showing dissimilarity among genotypes.

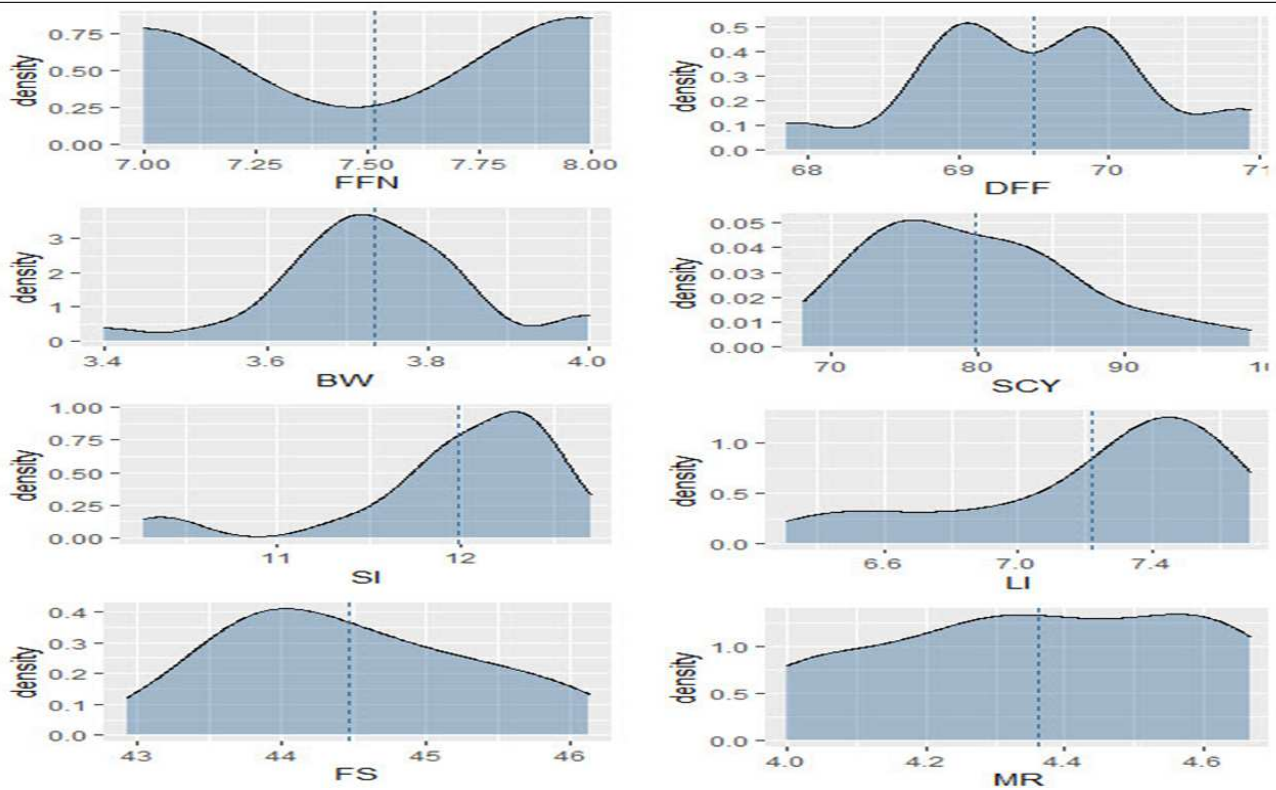


Figure 4. Density plots of the studied traits.

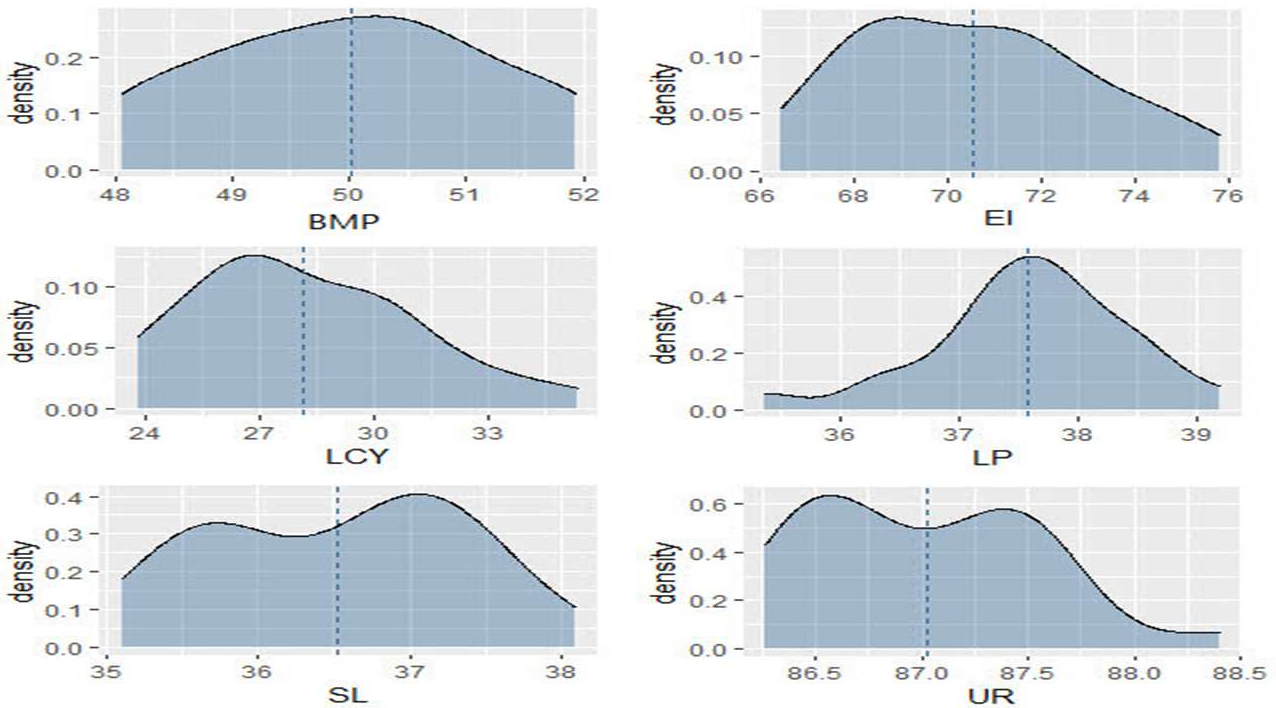


Figure 4. continued Density plots of the studied traits.

DISCUSSION

The findings of this study underscore the significant role of intermating and recurrent selection in cotton breeding programs, offering valuable insights into the genetic architecture and potential for genetic enhancement in cotton varieties. Intermating and recurrent selection could be considered more important to select superior families in the next generation of breeding programs. Of most of the studied traits in both biparental selection cycles (BIPS1 and BIPS2) were revealed in the desired direction in the BIPS2 population. This performance may be due to the pooling of desirable alleles through recombination in BIPs2 and the possibility of breaking down some relationships among the studied traits; these results were supported by those of (Dolan *et al.*, 2021; Ergashovich *et al.*, 2020). Estimates of coefficient of variation exhibited differences between GCV and PCV for earliness index, seed cotton yield, lint cotton yield, seed index and lint index traits. It may be attributed to the significant magnitude of additive and environmental effects (Devi, Prasad, and Rao 2022; Dianga, Joseph, and Musila 2020). Some relationships among features changed from BIPS1 to BIPS2 in value and direction. In BIPS2, the correlation value between seed cotton yield and lint index went from being non-significant to being positive (-0.151 to 0.445). In BIPS1, this correlation value was non-significant. This section should not be used to repeat the findings; rather, the results should be explained here using reasoning and

referencing relevant preceding literature. It is not necessary to present every result and re-repeat every value at this time. Rather, a logical and scientific explanation would encourage intercrossing and recurrent selection in early segregating generations in such self-pollinated crops as wheat to break unfavorable linkages to retain greater variability for several selections. This aimed to maintain greater genetic diversity. There was an apparent increase in seed cotton output associated with high mean values of the self-pollinated earliness index (BIPS2). The top five families picked had earliness indexes ranging from 23.951% to 19.308%, and seed cotton yields ranging from 55.193% to 36.233%. These ranges were also seen across the board. As shown by these data, many families, such as No. 5, No. 7, No. 8, No.18, and No.19, displayed large yields and early maturation. It's possible that this is because to the genetic association that exists between early maturity and seed cotton output (0.998%). Therefore, the selection for these families, which are distinguished by high yield and early maturity in succeeding generations, will be successful. Because of this, continued selection, and assessment for these BIPS families on a broad scale may be useful depending on how well they function. This will improve the possibility that they will derive genotypes that have desired traits. In conclusion, the findings of this study contribute to our understanding of cotton breeding strategies and emphasize the importance of intermating and recurrent selection in achieving genetic improvement. Moving forward, future research could explore additional aspects

of these breeding methods, such as marker-assisted selection and genomic selection to further optimize cotton breeding programs for enhanced yield and fiber quality. By integrating cutting-edge technologies with traditional breeding approaches, cotton breeders can accelerate the development of improved varieties that meet the evolving needs of the global cotton industry.

Conclusions: The findings of this study underscore the effectiveness of biparental selection cycles (BIPS1 and BIPS2) in improving key traits relevant to cotton breeding programs. Through recombination of desirable alleles and disruption of unfavorable genetic linkages, the BIPS2 population demonstrated a notable shift towards desirable trait performance. Specifically, fiber traits such as fiber length, strength, and micronaire reading showed significant improvements, indicating the potential for enhancing fiber quality alongside yield. The observed variations in coefficient of variation highlight the complex interplay between genetic and environmental factors in trait expression, emphasizing the importance of careful selection for trait improvement. Moreover, the higher heritability estimates in BIPS2 families signify the presence of substantial genetic variation that can be leveraged for future breeding endeavors. These results align with previous research and underscore the potential of biparental selection strategies in maintaining and enhancing genetic diversity over successive generations. Moving forward, the insights gained from this study pave the way for more targeted breeding efforts aimed at further improving cotton varieties. By focusing on specific fiber traits that exhibit promising improvements, breeders can expedite the development of high-yielding and resilient cotton cultivars. Additionally, continued exploration of genetic correlations and observed gains will inform future breeding strategies, ensuring sustained progress in cotton breeding programs.

Ethics declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Conflict of interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table S1. Observed direct gain and correlated response for seed cotton yield, earliness index measured in percentage of the biparental selfed pollinated2 (BIPS₂), of the better parent.

Family	SCY % BIPS ₂					EI% BIPS ₂				
	BIPS ₂ -7	BIPS ₂ -8	BIPS ₂ -19	BIPS ₂ -18	BIPS ₂ -5	BIPS ₂ -7	BIPS ₂ -19	BIPS ₂ -8	BIPS ₂ -5	BIPS ₂ -18
BMP	-23.766**	-20.916**	-24.987**	-24.987**	-24.987**	-23.766**	-4.987	-20.916**	-24.987**	-24.987**
EI %	23.951**	21.564**	21.755**	19.308**	20.283**	23.951**	21.755**	21.564**	20.283**	19.308**
BW(g)	12.371*	16.701*	12.371	17.938*	17.526*	12.371*	12.371*	16.701*	17.526*	17.938*
SCY/P(g)	55.193**	45.557**	44.905**	36.233**	35.511**	55.193	44.905**	45.557**	35.511**	36.233**
LCY/p(g)	61.923**	51.678**	49.128**	39.941**	37.589**	61.923	49.128	51.678	37.589**	39.941
L %	10.126**	9.854**	8.719**	9.088**	7.837**	10.126**	8.719**	9.854**	7.837**	9.088**
SI(g)	13.545*	17.406**	16.583**	18.355**	13.924*	13.545*	16.583**	17.406**	13.924*	18.355**
LI(g)	10.914**	14.416**	11.827**	13.959**	7.614**	10.914**	11.827**	14.416**	7.614**	13.959**
2.5%SL (mm)	2.002	6.292**	2.383**	8.961**	1.525	2.002	2.383**	6.292**	1.525	8.961**
UR	1.402*	2.376**	2.298**	2.376**	2.259**	1.402*	2.298**	2.376**	2.259**	2.376**
FS (g/tex)	1.869**	6.542**	3.427**	3.115**	2.181**	1.869**	3.427**	6.542**	2.181**	3.115**
MR	6.475	13.669*	0.000	-0.720	7.194	6.475	0.000	13.669*	7.194	-0.720
BMP	-24.987**	-22.341**	-26.412**	-24.885**	-21.018**	-23.461**	-24.885**	-22.443**	-20.916**	-23.359**
EI %	19.308**	13.984**	17.172**	17.406**	16.578**	11.095	17.406**	12.109**	21.564**	16.578*
BW(g)	17.938*	14.433*	15.464*	14.845*	19.175*	17.320*	14.845*	12.165*	16.701*	16.082*
SCY/P(g)	36.233**	19.671	30.819**	31.863**	30.203**	17.290	31.863**	7.241	45.557**	33.466**
LCY/p(g)	39.941**	22.700	38.484**	39.025**	36.768**	21.468	39.025**	9.633	51.678**	38.493
L%	9.088**	9.661**	12.638**	12.221**	11.804**	11.047**	12.221**	10.291**	9.854**	10.155**
SI(g)	18.355**	20.570**	12.026*	13.671*	16.077**	16.836**	13.671*	12.469*	17.406**	15.064**
LI(g)	13.959**	17.107**	13.452**	14.568**	16.193**	15.685**	14.568**	10.203*	14.416**	12.640**
2.5%SL (mm)	8.961**	7.626**	7.245**	7.245**	6.673**	4.576**	7.245**	5.720**	6.292**	5.148**
UR	2.376**	2.454**	2.143**	1.987*	1.013	1.441*	1.987*	2.065**	2.376**	1.091*
FS(g/tex)	3.115**	4.361**	3.738**	7.477**	4.361**	7.788**	7.477**	6.854**	6.542**	5.919**
MR	-0.720	13.525*	11.511*	13.669*	3.597	-0.720	13.669*	-0.720	13.669*	0.719

*and ** significant at 0.05 and 0.01 levels of probability, respectively