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Genetic Diversity in Elite Cotton Germplasm Lines Using Field Performance and Rapd Markers

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Abstract: The field experiments of the plant materials used in this investigation was carried out in Egypt during (2002-2005) while, 21 cotton genotypes were subjected to RAPD analysis using 53 UBC 10-mer RAPD primers in (2006) in Lab of Cotton Genetics and Breeding (Department of Agronomy and Horticulture) New Mexico State University, USA. Twenty three primers showed good amplification of cotton genome DNA. A total of 113 scorable bands were detected, among which 96 bands (84.95%) were polymorphic. A dendrogram constructed from the RAPD data classified the 21 cotton genotypes into four major clusters. L_1 was alone in a separate group (A) as the most divergent genotype, followed by L_{15} in the second group (B).The third cluster © included 13 genotypes and was divided into five sub-clusters, the fourth one (D) included 6 genotypes and divided into three sub-clusters. Results from field experiments revealed large variability for yield and its components between tested genotypes. High broad sense heritability estimates were detected for all traits studied. The cluster analysis based on Euclidean distance using yield characters grouped the 21 cotton genotypes into two main groups at 20 Euclidean distances. Cluster "A" and "B" composed of eleven and ten genotypes, respectively. Highest seed cotton yield per plant and its components was recorded in the three outstanding lines L_{10}, L_{16} and L_{18}. These lines were grouped in the same sub-sub group (B11) based on yield characters (more closely related) however, based on RAPD analysis, the three lines were classified into different sub-clusters (more genetically distant). This experiment demonstrated that simultaneously RAPD analysis and yield evaluation are useful for characterizing genetic diversity and defining relationships between cotton germplasm lines.

Key words: *Gossypium barbadense* L · Genetic diversity · RAPD analysis · Cluster analysis · Similarity Coefficients · Heritability

INTRODUCTION

The lack of genetic diversity is implicated in the slowing of progress in developing new cotton cultivars with improved yield and quality potential, as well as stress resistance. In order to broad the cotton genetic base, this may be accomplished by collection of available germplasm or developing inter - and intra-specific hybrids. The inter-specific hybrids are not always successful because the occurrence of genetic breakdown in the F2 and subsequent generations after obtaining the fertile F1's. Therefore, many programmes are interested to develop intra-specific hybrids which will, to a great extent, depend upon the genetic diversity prevalent in the existing population. The introgression of new genetic materials (genes) through intra-specific hybridization into new released cultivars will increase the cotton genetic base.

Successful breeding program depends on the complete knowledge and understanding of the genetic diversity within and among genetic resources of the available germplasm and enable plant breeders to choose parental sources that will generate diverse populations for selection.

Several methods have been used to estimate the genetic variability in cotton. Molecular markers will be useful in reducing the size of populations by evaluating the materials at early stages. RAPD has been successfully used in identification and differentiation between different cotton cultivars. Many investigators studied the genetic diversity in cotton genotypes using DNA (RAPD) procedure [1-6].

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The objectives of the present study were, to evaluate the agronomic characters of 21 cotton genotypes, i.e. 19 F5 lines derived from intra-specific hybridization between Giza 70 (Egyptian variety) and S.8017 (the Uzbek. zero branching variety) under field conditions, detect the genetic divergence between genotypes based on agronomic characters and to assess the level of genetic diversity and genetic relationship among them in the F6 using RAPD molecular markers procedure.

MATERIALS AND METHODS

Field Experiment: The material used was F2 plants which originated from the G. barbadense cross Giza 70 x S. 8017 (an early Uzbek. zero branching variety). This cross was the most promising out of forty five crosses evaluated by Esmail [7]. Four cycles of selection were applied (2002, 2003, 2004 and 2005) with artificial selfing the selected plants, following the traditional pedigree selection. Progenies of 200 F2 plants were grown in 3 m long rows with row to row and hill to hill spacing of 70 and 25 cm, respectively. At harvesting 3 plants per row were visually selected from the best rows based on their yielding ability and early maturity. The progeny rows were applied to test the performance of the selected lines in the next generations. The same procedure was repeated for three more cycles. The best 19 lines having both good yielding ability, early maturity and combined the favorite traits from their parents, were selected and further evaluated with the two parental lines in the F5 generation during 2005. The 21 genotypes (19F5 lines plus the two parents) were grown in three rows plots in 2005 at the Experimental Research Station of the National Research Centre at Shalakan El-Kalyoubia Governorate, Egypt. Rows length was 3 m and the row spacing was 70 cm. Plant spacing within the row was 25 cm between plants. The plots were un-replicated. At maturity, fifteen competitive plants from each genotype were randomly selected and scored the following traits: Number of open bolls per plant, (OP/P), Boll weight (BW), Seed cotton yield per plant (SCY/P), Lint cotton yield per plant (LCY/P) and Lint percentage (LP%). Analysis of variance was performed on all F5 data using the general linear model procedure of SAS [8]. Data processing was performed using SPSS computer software [9], in order to assort genotypes according to their agronomic characters.

RAPD Fingerprinting: The 21 cotton genotypes tested were grown in the greenhouse in 2006 and young leaves from each genotype were harvested. Total cellular DNA from the young cotton leaves (age 19 days) was extracted and cleaned-up using cTAB-based mini-prep method developed for Gossypium species and cultivars [10]. RAPD products (random amplified segments of DNA) were generated by 10-mer primer sets ordered from the University of British Colombia (UBC). The RAPD-PCR reactions were carried out in an ABI Gene Amp PCR System (9700 or 2720) thermal cycler in 26 μL at Cotton Genetics and Breeding Lab at the Department of Plant and Environmental Sciences (formerly Department of Agronomy and Horticulture) at New Mexico State University, USA. RAPD reaction mixtures were in 26 μL, 18.4 μL diH_2O, 2.5 μL 10 X PCR buffer Π, 1.5 μL 25 mM $MgCl_2$, 0.5 μL dNTP, 1.0 μL UBC RAPD primer, 0.1 μL Taq DNA polymerase and 2.0 μL genomic DNA. The reactions were at 94°C for 3 min, then 45 cycles at 94°C for 1 min, 1.5 μL 25 mM $MgCl_2$, 0.5 μL dNTP, 1.0 μL UBC RAPD primer, 0.1 μL Taq DNA polymerase and 2.0 μL genomic DNA. The reaction were at 94°C for 3 min, then 45 cycles at 94°C for 1 min, 40°C for 3 min, 72°C for 1.3 min, followed by 72°C for 5 min.

Polymerase chain reaction (PCR) products were separated by electrophoreses in 1.2% agarose gel using 1X TBE buffer and visualized by ultraviolet illumination after stained with ethidium bromide. Amplification profiles off all 21 cotton genotypes were compared with each other and bands of DNA fragments were scored as a binary variable with (1) for presence and (0) for absence. Genetic similarity coefficients were calculated on the basis of simple much coefficients (SM) and Jaccard's coefficient (JC) using the Numerical Taxonomy Multivariate Analysis System (NTSYSpc) Version 2.1 software package. The resulting similarity coefficients were used to perform the cluster analysis by the unweighted pair group method of arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) based marker technique that has been used for estimation of genetic diversity of populations and for studying the genetic relationships between different genotypes.

In this investigation 21 cotton genotypes were subjected to RAPD analysis using 53 UBC 10-mer RAPD primers. Twenty three primers showed good amplification of cotton genome DNA. A total of 113 scorable bands were detected, among which 96 bands (84.95%) were polymorphic with the mean of 4.17 per primer, while the remaining were monomorphic in nature. The percentage of

Table 1: Size and number of amplified and polymorphic bands obtained by PCR of DNA from cotton genotypes using 23 UBC RAPD primers

Primers	Size (bp)	Amplified bands	Polymorphic bands	Percent of polymorphism
UBC 17	500-1400	6	6	100
UBC 18	600-1500	6	6	100
UBC 19	450-1350	6	5	83
UBC 20	650-1400	5	5	100
UBC 23	450-1450	6	6	100
UBC 25	450-1500	4	4	100
UBC 28	350-1000	5	5	100
UBC 38	750-1250	5	4	80
UBC 48	600-1100	5	4	80
UBC 53	600-1500	5	5	100
UBC 54	650-1500	4	3	75
UBC 55	500-1200	4	1	25
UBC 56	600-900	2	1	50
UBC 57	550-1100	4	2	50
UBC 59	450-1500	5	5	100
UBC 60	500-1500	5	3	60
UBC 60	350-1500	7	7	100
UBC 62	800-1450	4	3	75
UBC 64	500-1500	8	7	87
UBC 65	650-1500	4	2	50
UBC 66	500-1450	4	4	100
UBC 67	100-1500	4	4	100
UBC 68	100-1000	5	4	80
Total	==	113	96	===
average	==	4.91	4.17	84.95

polymorphic markers varied from 25 to100%, Table 1 and Fig. 1, however polymorphism levels differed from one primer to the other. The number of bands for each primer ranged from 2 to 8, with an average of 4.91. The size of the amplification products ranged between 100-1500 bp. Rubeena and Randhawa [3] found the size of the amplification products ranged between 201 and 2888 bp. The highest number of 8 RAPD markers were produced by UBC 64, followed 7 markers by UBC 61 while, UBC 61 showed high polymorphic (100%) than UBC 64 (87%).

Determining true genetic dissimilarity between individuals is an important and decisive point for clustering and analyzing diversity within and among populations, because different dissimilarity indices may yield conflicting outcomes [11]. The similarity coefficient values among the studied cotton genotypes based on RAPD analysis are presented in Table 3. From our results, it is obvious that the most similar lines were L_9 and L_{10}, with similarity coefficient (0.92). On the other hand, L_1 followed by L_{15} are the most dissimilar from the others. This result indicated that these derived lines showed significant genetic differentiation. Although, the 19 derived lines were descended from common ancestors, an average genetic distance was detected between them, similarity coefficients ranged from 0.58 to 0.92. This is expected because of the parental varieties (Giza 70 and S.8017) were different in their geographic origin and pedigrees. Zhang *et al.* [12] found DP555BR and DP449BR shared cv. DP5690 in their pedigree but they were grouped separately and they concluded that pedigree information or geographic origins of cultivars may not accurately reflect genetic relatedness among genotypes, whereas DNA markers could better reveal the genotypic relationships when there are sufficient markers and they are distributed across all chromosomes. Using RAPD markers [13], who found that low genetic distance (0.01 to 0.08) between nine Australian cotton cultivars also, Iqpal *et al.* [2] found low genetic distance (0.07 to 0.18) between 17 G. hirsutum cultivars. However, using SSR

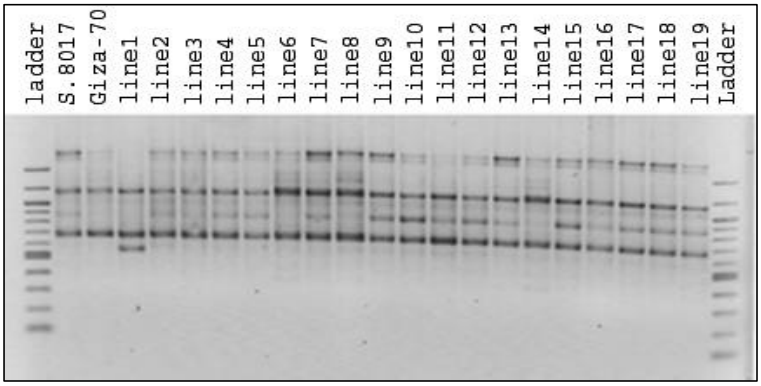

Fig. 1: Amplification RAPD profiles of 21 cotton genotypes with primer UBC-57. Ladder=100 bp DNA ladder used as molecular weight marker

Table 2: Genetic similarity based on Jaccard,s coefficient between cotton genotypes according to RAPD analysis

Genotypes	P1	P2	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19
S.8017	1																				
Giza70	0.89	1																			
L1	0.64	0.67	1																		
L2	0.78	0.81	0.73	1																	
L3	0.83	0.67	0.72	0.85	1																
L4	0.85	0.86	0.72	0.88	0.91	1															
L5	0.78	0.80	0.63	0.83	0.79	0.86	1														
L6	0.83	0.84	0.59	0.70	0.80	0.80	0.79	1													
L7	0.81	0.84	0.65	0.81	0.85	0.85	0.81	0.85	1												
L8	0.89	0.88	0.71	0.85	0.86	0.87	0.79	0.78	0.87	1											
L9	0.84	0.81	0.73	0.87	0.84	0.88	0.83	0.73	0.83	0.9	1										
L10	0.81	0.81	0.76	0.84	0.86	0.89	0.82	0.74	0.84	0.87	0.92	1									
L11	0.8	0.77	0.73	0.79	0.79	0.84	0.84	0.74	0.78	0.79	0.82	0.86	1								
L12	0.82	0.79	0.68	0.82	0.82	0.88	0.82	0.72	0.82	0.85	0.87	0.88	0.87	1							
L13	0.77	0.80	0.60	0.75	0.81	0.80	0.77	0.74	0.83	0.79	0.79	0.79	0.77	0.81	1						
L14	0.82	0.82	0.59	0.73	0.80	0.81	0.75	0.83	0.82	0.80	0.77	0.77	0.77	0.80	0.83	1					
L15	0.76	0.77	0.61	0.77	0.77	0.80	0.77	0.75	0.82	0.76	0.78	0.78	0.79	0.80	0.76	0.74	1				
L16	0.75	0.77	0.64	0.81	0.78	0.81	0.79	0.74	0.84	0.83	0.83	0.82	0.79	0.81	0.77	0.75	0.79	1			
L17	0.79	0.78	0.62	0.82	0.77	0.81	0.78	0.74	0.78	0.80	0.83	0.78	0.83	0.79	0.80	0.76	0.79	0.85	1		
L18	0.78	0.76	0.64	0.84	0.80	0.82	0.78	0.71	0.84	0.86	0.87	0.83	0.78	0.83	0.80	0.79	0.77	0.84	0.83	1	
L19	0.75	0.73	0.58	0.81	0.77	0.82	0.81	0.72	0.77	0.81	0.79	0.76	0.76	0.83	0.76	0.76	0.75	0.81	0.8	0.85	1

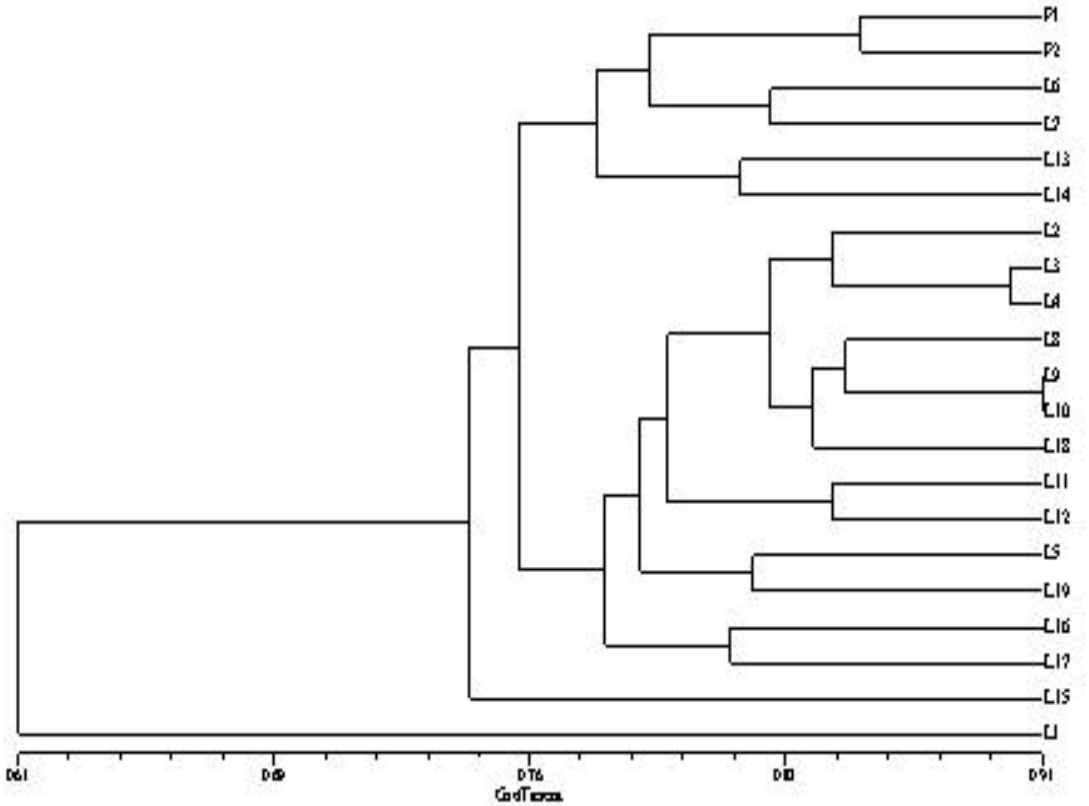

Fig. 2: A dendrogram of 19 elite germplasm cotton and their parents developed from RAPD data using the un weighted pair group method of arithmetic means (UPGMA) based on Jaccard's coefficient

Table 3: Mean square from analysis of variance of 21 cotton genotypes and broad sense heritability for all traits studied

S.O.V	D.F	(OP/P)	(BW) (g)	(SCY/P) (g)	(LCY/P) (g)	(LP %)
Lines Error	20	179.36**	0.754**	1169.58**	134.17**	20.03**
	294	31.33	0.103	233.27	26.62	3.09
h2 (b)	==	82.53	86.34	80.05	80.16	84.57

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

markers [14] who also found that narrow genetic distance (10 to 22%) between Australian and American cotton cultivars. Abdalla *et al.* [15] using AFLP and reported that the mean genetic similarity within our geographically and historically diverse set of *G. barbadense* accessions (0.89) was similar to our *G. hirsutum* collection (0.86). Zhang *et al.* [12] found sufficient genetic diversity when test a sample of elite commercial cotton cultivars (24 genotypes) including many transgenic cultivars, Jaccard's genetic similarity coefficients ranged from 0.694 to 0.936, with an average of 0.772 based on SSR markers. Rahman *et al.* [16] stated that, genetic relatedness among the elite cotton cultivars ranged from 81.41 to 94, 90%. Plant breeders select breeding material to breed for elite lines on the basis of genetic relationship among the breeding material [17].

The dendrogram generated from genetic distance coefficient, classified the 21 cotton genotypes into four major clusters (Fig. 2). L_1 was alone in a separate group

Table 4: mean performance of 19 F5 lines and their parents for agronomic traits studied

Genotypes	(OP/P)	(BW) (g)	(SCY/P)(g)	(LCY/P)(g)	(LP %)
L1	17.67 a-c	2.26 j	39.53 b-e	13.88 b-f	34.34 b-g
L2	12.33 e-g	2.56 d-h	31.39 e-g	10.63 f-h	3 3.29 f-I
L3	10.26 g	2.52 e-i	26.24 g	8.95 h	34.91 bc
L4	13.04 d-g	2.30 i-j	31.16 e-g	10.28 f-h	32.99 h-j
L5	15.87 b-e	2.42 g-j	38.26 c-f	12.18 c-h	31.76 j
L6	11.87 e-g	2.41 g-j	28.79 e-g	10.71 e-h	37.31 a
L7	12.13 e-g	2.67 c-f	32.03 e-g	10.78 e-h	33.72 c-I
L8	13.20 d-g	2.50 e-I	33.33 d-g	11.04 e-h	33.14 g-I
L9	15.06 c-e	2.63 c-g	39.22 b-e	13.24 c-g	33.62 d-I
L10	19.20 a-b	2.64 c-g	51.66 a	17.27 ab	33.44 e-I
L11	11.0 fg	2.86 a-c	32.02 e-g	11.13 e-h	34.83 b-d
L12	16.60 b-d	2.93 a	49.66 ab	16.99 ab	34.10 b-h
L13	9.53 g	2.91 ab	27.45 fg	8.93 h	32.51 ij
L14	14.93 c-f	2.94 a	43.55 a-d	14.41 b-e	33.0 h-j
L15	18.0 a-c	2.45 f-j	45.15 a-c	15.54 a-c	34.21 b-h
L16	21.6 a	2.39 h-j	51.28 a	17.55 ab	34.25 b-h
L17	18.80 a-c	2.38 h-j	44.43 a-c	14.99 a-d	34.41 b-f
L18	18.46 a-c	2.76 a-d	53.63 a	18.25 a	34.62 b-e
L19	10.60 g	2.70 b-e	28.99 e-g	9.68 gh	33.12 g-I
Giza70	11.93 e-g	2.43 g-j	28.82 e-g	10.27 f-h	35.28 b
S.8017	11.93 e-g	2.98 a	35.36 c-g	11.82 d-h	33.57 d-I
X	14.49	2.6	37.71	12.78	33.92
L.S.D.0.05	4.02	0.23	10.97	3.71	1.26

Means followed by the same letter do not differ significantly at the 0.05 level

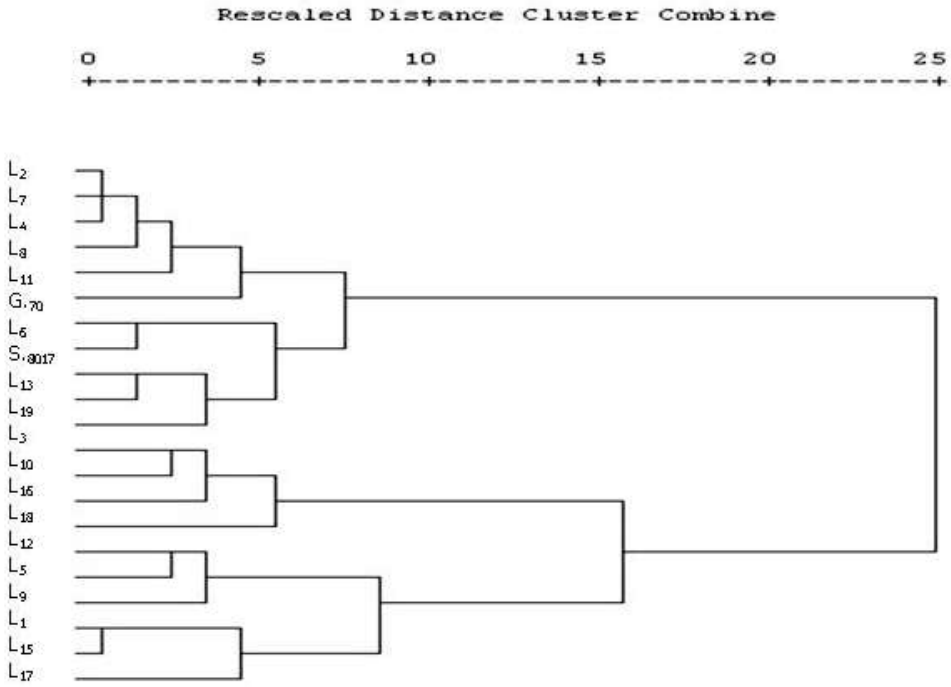

Fig. 3: Cluster diagram for 21 cotton genotypes classified by agronomic characters

(A) as the most divergent genotype, followed by L_{15} in the second group (B). Generally, two parental lines were distributed in one sub-cluster. Though, the distribution of 19 F6 lines was not grouped together into one cluster, indicating that a considerable genetic divergence was induced (resulting) by hybridization between the two parents and could be used as a sources for new cultivars development in cotton breeding programs. The third cluster (C) included 13 genotypes and was divided into five sub-clusters. The first sub-clusters included L_2,L_3 and L_4, the second sub-cluster included L_9, L_{10}, L_8 and L_{18}. In the third sub-cluster L_{11} and L_{12} formed a sister group relationship. Similarly, the fourth and fifth sub-clusters contained L_5 and L_{19}, L_{16} and L_{17}, respectively. The fourth cluster (D) included 6 genotypes and divided into three sub-clusters, was constituted of the two parents, L_6 and L_7, L_{13} and L_{14}. These results indicated that these new materials could probably be a source of genetic variability for cotton breeding programs. High levels of similarity within sub-clusters of G. barbadense group were found by Abdalla *et al.* [15]. While, Bertini *et al.* [18] studied the genetic diversity and found the dendrogram of the relationship between the 53 cotton cultivars belong to *G. hirsutum* were distributed into two large groups and seven well–nested subgroups, the majority of group (A) were obtained by selection, the group (B) cultivars were produced by crossing.

Results from field experiments of agronomic traits studied revealed that the mean performance exhibited a wide variation among tested genotypes for all traits studied (Table 4). Also, large variability for yield and its components was observed between tested genotypes. Highest seed cotton yield per plant and its components was recorded in L_{18}, L_{10} and L_{16}. Analysis of variance for means of the studied traits of all genotypes is presented in Table 3. The data revealed that mean squares due to genotypes were highly significant for all studied traits, indicating the existence of relatively large genetic variability among the studied characters. High broad sense heritability estimates were detected for all traits studied, indicating that these traits could be improved through pure line selection (Table 3). Esmail *et al.* [19] and Kale *et al*. [20] found similar results. Hendawy [21] found relatively low heritability value for seed cotton yield in the two cotton crosses (46.32% and 49.42%). Also, low heritabilty with low genetic gain was found for seed cotton yield per plant in the barbadense cross (2.27 and 1.7), indicating slow progress through selection for this trait [22]. However, Mahros [23] found moderate to high broad sense heritability estimates for seed cotton yield and most of its components in the three cotton crosses.

The genetic divergence can provide visual idea about variabilities presented in studied genotypes in addition to assuring the continued genetic improvement [24]. The cluster analysis based on Euclidean distance (tree diagram) using yield characters among cotton genotypes are illustrated in Fig.3. Clustering of 21 cotton genotypes produced two main groups at 20 Euclidean distances. Cluster "A" and "B" composed of eleven and ten genotypes, respectively. Two sub-groups within each group were detected. The first sub-group (A1) included L_2, L_7, L_8, L_4, L_{11} and the Egyptian cultivar Giza 70, were similar in lowest production of seed cotton yield per plant. While the second sub-groups (A2) included L_3, L_6, L_{13}, L_{19} and the Uzbek variety S.8017, were similar in boll numbers and lint yield per plant. On the other hand, group "B" divided into two sub-groups i.e. (B_1) and (B_2) composed of 4 and 6 genotypes, respectively. Both of them were divided into two sub sub-group, the first one (B_{11}) included L_{10}, $_{16}$ and L18 were similar in highest seed cotton yield per plant and its components. Sub sub-group (B_{22}) which included 3 lines no. 14,15 and 17 were lower in seed cotton yield than (B_{11}) sub sub-group. These outstanding lines should be exploited in the future breeding programs to develop new cotton cultivars that possess high yield potential or broad a cotton genetic germplasm base.

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