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التباعد الوراثي بين الأصول الوراثية للقطن وعلاقتها بتطور الأصناف باستخدام الصفات المحصولية وتقنية الـSSR

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الملخص العربي

يعتبر دراسة التباعد الوراثي والعلاقات الوراثية بين التراكيب الوراثية المختلفة من الأهمية لمربى القطن وعلى هذا أجرى هذا البحث بمحطة البحوث الزراعية بسخا وتم استخدام ٢٨ تركيب وراثي للقطن على أساس دراسة صفات معدل الحليج ، ومعامل الشعر وقراءة الميكرونير ، قيمة البرسيلى ، وطول التيلة ، درجة الاصفرار ، وإجراء تحليل الـSSR لهذه التراكيب المختلفة .

وكانت أهم النتائج المتحصل عليها كالتالي :

١- أظهر تحليل التباين وجود اختلافات معنوية بين التراكيب الوراثية المختلفة لكل الصفات تحت الدراسة .

٢- أوضح التحليل المتعدد باستخدام تحليل التمايز Discriminant function analysis أن الثلاث عوامل الأولى كانت معنوية وتمثل ٨٠.٤% من التباين الكلى بين التراكيب الوراثية .

٣- كما أوضح التحليل أن العامل الأول يمثل ٦٤.٦% من التباين الكلى وكان أكثر تأثراً بصفات معامل الشعر يتبعها قراءة الميكرونير ، بينما كان العامل الثاني Second function يمثل ١٥.٨% والثالث ١٢.٢% من التباين الكلى .

٤- تم توزيع التراكيب الوراثية (٢٨تركيب) على أساس الثلاث عوامل الأولى في عشرة مجاميع رئيسية .

٥- تراوحت قيم Euclidean distance بين ٠.٥ بين الصنفين المنوفى وجيزة ٦٨ إلى ٦٠.٨ بين الصنفين جيزة ٤٥ وجيزة ٨٠ .

٦- كما تم توزيع التراكيب الوراثية على أساس عدم التشابه النسبى بينها على إحدى عشر تجمع حيث كانت أكبر مسافة داخل التجمع رقم VIII والتجمع رقم II واللذان يحتويان على ٦ ، ٢ تراكيب وراثية على التوالي ، بينما كانا التجمع رقم III والتجمع رقم V أكثر تباعداً فيما بينهما ، وأظهر التجمع رقم VII ، IX علاقة قرابة نسبية.

٧- أظهرت ٤٤ حزمة من بين ٥٢ حزمة من حزم الـSSR اختلافات بين الأصناف أي ما يقرب من ٨٤.٦% من الحزم الكلية ، بينما أظهر تحليل التشابه على أساس نتائج SSR أن معامل التشابه Similarity cofficient يقع بين ٨٨.٩% إلى ٣٨.٥% .

٨- تم توزيع الـ٢٨ تركيب وراثى إلى ٩ مجموعات رئيسية على أساس عدم التشابه النسبى لنتائج الـSSR

كما أوضحت النتائج أن القياسات المحصولية واستخدام الـSSR متكاملان لدراسة وإيضاح التباعد الوراثى والعلاقة بين أصول القطن المصرى ، مما يفيد الحفاظ على صفوة السلالات والتراكيب الوراثية الجيدة وتطور برامج التربية المستقبلية .

COTTON GERMPLASM DIVERSITY AND ITS RELATION TO VARIETAL IMPROVEMENT AS REVEALED BY AGRONOMIC CHARACTERS AND SSR MARKERS TECHNIQUE .

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ABSTRACT: *Genetic diversity and relationships between genotypes are great importance for Cotton breeding. Twenty eight Cotton genotypes belonging to G.barbadense were analyzed for genetic diversity using Six agronomic Traits and Six simple sequence repeat (SSR) microsatellite locus. Analysis of variance revealed highly significance difference for all studied characters. The First three canonical variat are significant. This accounted for 80.4% among genotypes variance. The first canonical discriminate function represented 64.6% of the total variance and dominated by a large loading from lint index followed by micronaire reading. While the second and third functions accounted for 15.8 and 12.2% of the total variance respectively. The 28 cotton genotypes were platted according to first three functions in ten groups .*

Squared Euclidean distance were ranged from 0.502 between Menoufi and Giza 68 to 60.815 between Giza 45 and Giza 80. The 28 Cotton genotypes were grouped into eleven cluster on the basis of dissimilarity coefficient and six agronomic characters. Maximum intra cluster distance was found in cluster VIII and II which consisted of Six and Two genotypes respectively. Clusters III and V have maximum distantly clusters while clusters VII and IX are closely related. Out of 52 bands generated from the SSR 44 bands were polymorphic accounting for 84.6% of the total number of generated bands. The similarity coefficients based on SSR markers ranging from 88.9% to 38.5%. Thus suggesting considerable genetic variation among the Cotton genotypes. Clustering of 28 Cotton genotypes based on SSR markers resulted in nine major clusters. Results from agronomic measurements and SSR markers are complementary factor for each other in studying the genetic divergence and relationships among genotypes and both gave essential information for understanding genetic diversity of Egyptian cotton germplasm. Furthermore they provided a useful guide for conserving elite cotton germplasm and developing future cotton breeding programs.

Key Words : *Cotton, Genetic Diversity, Genetic Relationships; Discriminate Function; Simple sequence repeat (SSR) marker.*

INTRODUCTION

Knowledge of genetic diversity and relationships among breeding materials is essential to the cotton breeder for efficient improvement of cotton crop. Analysis of genetic divergence has been used by breeders for more than three decades to classify genotypes. Mainly this was done to maintain genetic diversity to be able to choose the desired hybrid combination .

Although it is widely assumed that genetically diverse parents facilitate the creation of superior progeny, few studies have examined the relationship between parental genetic distance and the creation of successful varieties. In theory, mating of distantly-related parents will produce a greater number of transgressive segregates than mating of closely-related parents. Cornelius and Sneller (2002) reported that a lack of genetic diversity may limit breeding progress. Information of genetic diversity is important when working to improve crop and develop new varieties. Also, characterizing genetic diversity and/or degree of homogeneity between and within varieties would be the first step toward developing germplasm and crop cultivars. Successful crop improvement depends on genetic variability that arises from genetic diversity (Rana and Bhat, 2005).

However, for many crops, yield improvements have been realized by hybridization closely-related genotypes. Esbroeck and Bowman (1998) observed that parental genetic diversity, as estimated by coefficient of parentage, was not imperative for cotton improvement. Successful cultivars were most frequently developed from closely-related parents, with a level of diversity similar to the average genetic relationship among regionally-adapted cultivars. These indicated that there were sufficient variability or mechanisms to create variability, to make breeding progress in a narrow germplasm base. Unless methods are improved to transfer useful allelic variation from diverse to adapted germplasm without negative agronomic effects, cotton germplasm resources will remain largely underused and the trend towards increased genetic uniformity will probably continue.

Genetic variation may be measured by several ways considerable overlapping may occur in univariate analysis, since each variable is viewed separately (Vaylay and Santon, 2002). In canonical discriminate analysis all independent variables (traits) are considered simultaneously in the differentiation of cultivars. The resulting differentiation of populations is more distinct compared to univariate analysis. After extraction of the among population variability (genetic), the genetic differentiation between populations can be measured by Euclidean distances .

Simple sequence repeats (SSRs), also known as microsatellites, have been proven as an excellent tool for cultivar identification, pedigree analysis and the evaluation of genetic distances among plant species (Priolli *et al.,* 2002). These have been reported to detect high level of polymorphism even amongst closely related plant germplasm-SSR markers found to be more variable within genomes of olive than other molecular marker types (Dongre *et al.,* 2004). SSRs have become a tool of choice for investigations of genetic relatedness between accessions, and the assessment of genetic diversity contained within a collection due to their Co-dominant inheritance and amenability to high through put analysis (Hokanson *et al.,* 1998).

Our objectives were to use canonical discriminate analysis to study the differences among cotton genotypes and to ascertain the magnitude of genetic divergence among 28 cotton genotypes to develop of successful cultivars. Moreover determine the genetic relationships using SSR markers. Such information may identify the breeding strategies that are most likely to produce improved progeny.

MATERIALS AND METHODS

1. Genetic Materials:

Twenty-eight *Gossipium barbadense* L. genotypes, selected on the basis of economical and historical importance as well as pedigree availability, were involved in this study. The genotypes included promising, commercial and ancient varieties to study the relationships in potentially new genotypes of Egyptian cottons. The pedigrees of the varieties are presented in Table (1).

The twenty-eight cotton genotypes used in this study were grown at Sakha Agric. Res. Station in 2011 season. The experimental design was randomized complete block with three replications. A plot consisting of 3 rows of 4.5 m long was used as an experimental unit. Rows were spaced 65 cm apart with a plant to plant distance of 25 cm. conventional practices were applied in the field. Data were recorded on 30 guarded plants basis for each entry for the following 6 characters: lint percentage (L %), lint index (LI), micronaire reading (MR), pressley index (PI), 2.5% span length (2.5% SL) and yellowness degree (+b).

Molecular markers (DNA isolation):

The twenty eight cotton genotypes were used for DNA isolation. They were kindly provided by the Agricultural Research Center, Cotton Research Institute, Sakha, Egypt. There pedigrees are presented in Table (1).

Table (1): Pedigrees of the 28 cotton varieties used in this study.

Genotype	Pedigree*	Genotype	Pedigree*
Giza 45	Giza 28 x Giza 7	Giza 88	Giza 77 x Giza 45-B
Giza 67	Giza 53B x Giza 30	Giza 89	Giza 75 x Russian-6022
Giza 68	menoufi x Giza 56	Giza 90	Giza 83 x Dendera
Giza 69	Giza 51A x Giza 30	Giza 92	Giza 84 x (Giza 74 x Giza 68)
Giza 70	Giza 59A x Giza 51B	Giza 89 x Giza 86	Giza 89 x Giza 86
Giza 75	Giza 67 x Giza 69	Giza 77 x Pima S_6	Giza 77 x Pima S_6
Giza 76	menoufi x Pima S_2	Giza 89 x Pima S_6	Giza 89 x Pima S_6
Giza 77	Giza 70 x Giza 68	Ashmouni (Giza 19)	Selected from Giza 2
Giza 80	Giza 66 x Giza 73	Dendera (Giza 31)	Selected from Giza 3
Giza 81	Giza 67 x H10867/63	Karnak (Giza29)	Maarad x Sakha 3
Giza 83	Giza 72 x Giza 67	Menoufi (Giza 36)	Wafeer x Sakha 3
Giza 84	Giza 68 x C.B.58	Pima S_2	American-Egyptian Varieties
Giza 86	Giza 75 x Giza 81	Pima S_6	
Giza 87	Giza 77 x Giza 45-A	6022	Russian variety

* Pedigree information from Abdel-Salam (1999).

Cotton seeds were grown in the green house and leaves of seedlings (after ten days of growth) were collected. Samples were directly grinded in liquid nitrogen using pestle and mortar. About 0.5 g of the grinded tissue was collected in 1.5 ml sterilized eppendorf tube. DNA isolation and purification was carried out using modified CTAB (Cetyl-tetramethyl ammonium bromide) method (Dellaporta *et al.,* 1983).

SSR analysis:

Six primer pairs specific for cotton microsatellite (SSR) were selected to carry out the SSR analysis and presented in Table (2) according to the literature (Hussein *et al.,* 2007 and Zhu *et al.,* 2003). The PCR amplification reactions were performed using 50 ng DNA at a 25 µl volume reaction containing 0.3 µmoles of each primer, 200 µM of dNTPs, 5 µl (1X) of Taq polymerase buffer, 1.5 mM MgCl2 and 0.5 U Taq DNA polymerase. The SSR reactions were carried out using Touchdown PCR program. The main program was: 9 cycles at 94^{o}C for 1 min, 54^{o}C for 1 min, decreasing 1^{o}C in every cycle, and 72^{o}C for 1 min, followed by 28 cycles at 94^{o}C for 1 min, 54^{o}C for 1 min and 72^{o}C for 1 min. The previous cycles were preceded by a denaturation step at 94^{o}C for 5 minutes and followed by an extension step at 72^{o}C for 5 minutes. PCR products were separated on 1.5% agarose gel electrophoresis.

2. Statistical analysis:

The data were subjected to the analysis of variance of all genotypes for every character separately. This analysis provides a test of significance between genotypes. After this step, multivariate technique (Hair *et al.,* 1987) was conducted by using: (i) Canonical discriminate analysis. This is a dimension-reduction technique related to principal component analysis and canonical correlation. given a classification variable, such as population or age group, and several quantitative variables. Furthermore the canonical discriminate analysis derives canonical discriminate functions (linear combinations of these quantitative variables) that highest possible multiple correlation with groups and summarizes among class variation. It facilitates differentiation of groups by taking into account the interrelationships of the independent variables (traits) and the dependent (cultivars). An important property of canonical variables is that they are uncorrelated even though the underlying quantitative variables may be highly correlated.(ii) Hierarchical clustering was then carried out on each data set using Ward's minimum variance method, which

minimizes within-cluster sum of squares. The results from clustering analysis are presented as dendrograms. The dendrogram is constructed on Euclidean distance basis. According to Anderberg (1973) and Nei (1973) and developed by Johnson and Wichern (1988).

All gels of the different molecular markers were scored as 0/1 for absence/presence of the bands, respectively and the resulting scored band sheets were analyzed using the TSYS-pc2.1 software (Rolhf, 1998). Similarity coefficient matrices were calculated for all the markers (mixed together) using simple matching similarity algorithm (Sokal and Sneath, 1963). Phylogenetic dendrogram was constructed using the UPGMA method (Sneath and Sokal, 1973). All these computation were performed using SPSS (1995) computer procedure.

RESULTS AND DISCUSSION

Estimated mean squares of the twenty-eight cotton genotypes for the studied characters are presented in Table (3). The results showed highly significant mean squares of all genotypes for the studied characters. The observed significant variation among the genotypes might reflect their different genetic background and this relying variability could be exploiting through hybridization program.

Univariate statistical techniques and analysis of variance do not show how cultivars or strain lines within cultivars differ when all variables are considered together. Canonical discriminate analysis simultaneously examines differences in the morphological variables and indicates the relative contribution of each variable to cultivar discrimination. Multivariate procedures based on morphological and agronomic characters have been used in the assessment of genetic divergence in Egyptian cottons.

In an analysis with six variables, six functions were existed. However, only those which exhibited high multivariate variations were considered. The first five functions accounted for all variation among genotypes.

The first two canonical variate were significant (P<0.01) and accounted for 80.4% of the among genotypes variance (Table 4). Each canonical variate is the linear combination of the independent variables (traits) and is orthogonal to the other. Canonical correlation measures the strength of the overall relationships between

Differentiation of genotypes

the linear composites of predictor (canonical discriminate variate) and criterion (genotypes) sets of variable. The significant (P<0.01) canonical correlation between the genotypes with the first and second canonical variates 0.985 and 0.943, respectively can explain the differentiation of the genotypes.

Table (2): Six selected SSR primers .

SSR Primers		
L11	AAAAACCCCTTTCCATCCAT	GGTGTCCTTCCCAAAAA
M8	GGCATCTTACGGTGGAAATGAC	GTTAGGTTTGGGGTGTTACATAC
M11	TGGACTAACCTAAACTTGACAC	CCTATGTACATATGCTCTTC
C2-0109	GTGAAAACCCGCAAAG	ATACCTAGTATTGCCCTTAT
C2-0119	GGTCCTTTTCGTCCTT	GGTATAAATATAATGATGGT
SSR3	GCACTCGAAGGAATTAATTTT	GAACAGTTGTTTCGTGTCGTA-3

Table (3): Mean squares of the studied characters for twenty-eight cotton genotypes .

S.O.V.	d.f.	L%	LI	MR	PI	2.5% SL	(+b)
Replications	2	0.379	0.031	0.029	0.028	0.112	0.840**
Genotypes	27	5.840**	1.020**	0.676**	1.212**	8.366**	8.843**
Error	54	0.317	0.033	0.028	0.229	0.376	0.105

** Significant at 1% probability level(P<0.01).

Table (4): The canonical loading of the independent variables for the first five canonical discriminate function of the cotton genotypes.

Variable[(a)]	Canonical discriminate function				
	1	2	3	4	5
Lint percentage (L%)	0.364	0.246	0.704*	-0.554	-0.074
Lint index (LI)	0.546	0.445	0.612*	-0.360	0.010
Micronaire reading (MR)	0.518	0.346	0.257	0.638*	-0.372
Pressley index (PI)	-0.181	-0.131	0.408	0.059	0.883*
2.5% span length (2.5% SL)	-0.466	-0.336	0.676*	0.153	-0.435
Yellowness degree (+b)	0.036	0.014	0.137	0.227*	0.059
Eigen value	32.789	8.033	6.090	3.331	0.514
% of variation	64.6	15.8	12.2	6.6	1.0
Cumulative %	64.6	80.4	92.4	99.0	100
Canonical correlation	0.985	0.943	0.927	0.877	0.583

(a) This variable not used in the analysis.

* Largest absolute correlation between each variable and any discriminate function.

Canonical loading measure the simple linear correlation between an original independent variable (traits) and canonical variate. Thus, the canonical loading reflecting the variance that the observed variable shares with the canonical variate, and it can be interpreted in assessing the relative contribution of each variable to each canonical function (Hair *et al.,* 1987).Each character was an important source of variation in, at least, one discriminate function. Some characters may have greater importance in determining plant phenotypes than others.

The first canonical discriminate function which represented 64.6% of the total variance among genotypes is dominated by a large loading from lint index followed by micronaire reading and 2.5% span length. The second function is largely dominated by lint percentage and accounted for 15.8% of a total variance (Table 4) among genotypes. While, the third is dominated by Presley index. Thus, it is evident that the genetic composition of the 28 cotton genotypes chiefly differed in these characters. Furthermore, each genotype could be plotted at the component score on each function. Each is a linear combination of the characters. The maximal amount of variance is shown on the first function and second maximal amount on the second function as well as third in the third function. The 3-dimensional distance between genotypes might reflect a summary of differences based on all characters measured to the extent that first three functions. The 28 cotton genotypes were plotted (Fig.1), according to the first three function, in ten groups. Most of these groups were among the genotypes at the same category and regretted from a common parent. Similar results were obtained by Vaylay and Santen (2002), Suinaga *et al.,* (2005), Hemaida *et al.,* (2006) and El-Mansy (2009).

From a plant breeding point of view, canonical discriminate analysis is useful in identifying the genetic variation and the most influential traits affecting genetic variation in plant population (Vaylay and Santon, 2002). Canonical loading of morphological and agronomic traits of an individual cultivar indicate the magnitude of genetic variation. The influential traits are the ones that change in response to natural selective forces.

Clustering of genotypes based on dissimilarity of characters

The actual values of Euclidean distances corresponding to the 378 possible comparisons showed that about 61.0% of values were significant. Squared Euclidean distances were ranged from 0.502 between Menoufi and Giza68 to 60.815 between Giza45 and Giza80. This was true, since the coefficient of parentage among Giza68 and Menoufi more than 75%. Based on Euclidean distances, the 28 cotton genotypes were grouped into eleven clusters with variable number of entries indicating the presence of considerable amount of genetic diversity in the material (Table 5) and (Fig. 2). These genotypes designated as the long stable, two groups, upper Egypt cottons , two cluster , extra long with extra fine, extra long with creamy lint, extra long with coarse lint and common parent, Ashmouni, as well as Karnak with Giza89 and the Russian variety 6022.

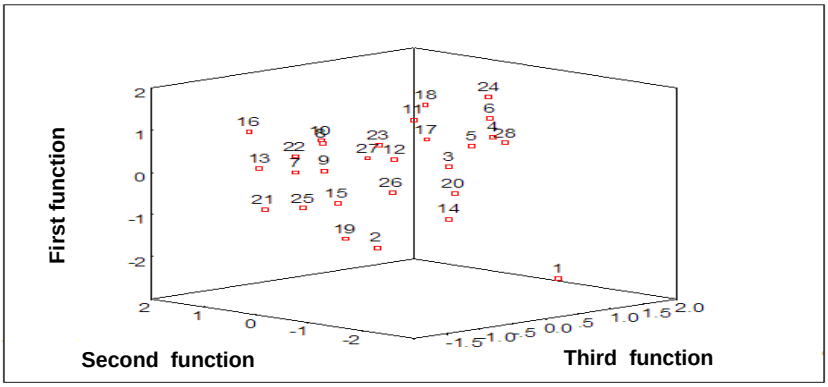

Fig.(1). 3-D. representation of intra cluster distance resulting from discriminate function analysis.

Table (5): Genotypes included in different clusters based on Euclidean distances

Clusters	No. of genotypes	Name of the genotypes
I	1	Ashmouni
II	2	Karnak , Giza 89
III	2	Dendera , Giza 80
IV	6	Menoufi , Giza 68 , Giza 77 x Pima S6 , Giza 88 , Giza 77 , Pima S6
V	2	Giza 45 , Giza 87
VI	3	Giza 69 , Giza 67 , Giza 89 x Pima S6
VII	1	Giza 70
VIII	6	Giza 75 , Giza 89 x Giza 86 , Giza 81 , Giza 84 , Pima S2 , Giza 86
IX	2	Giza 76 , Giza 92
X	2	Giza 83 , Giza 90
VI	1	6022

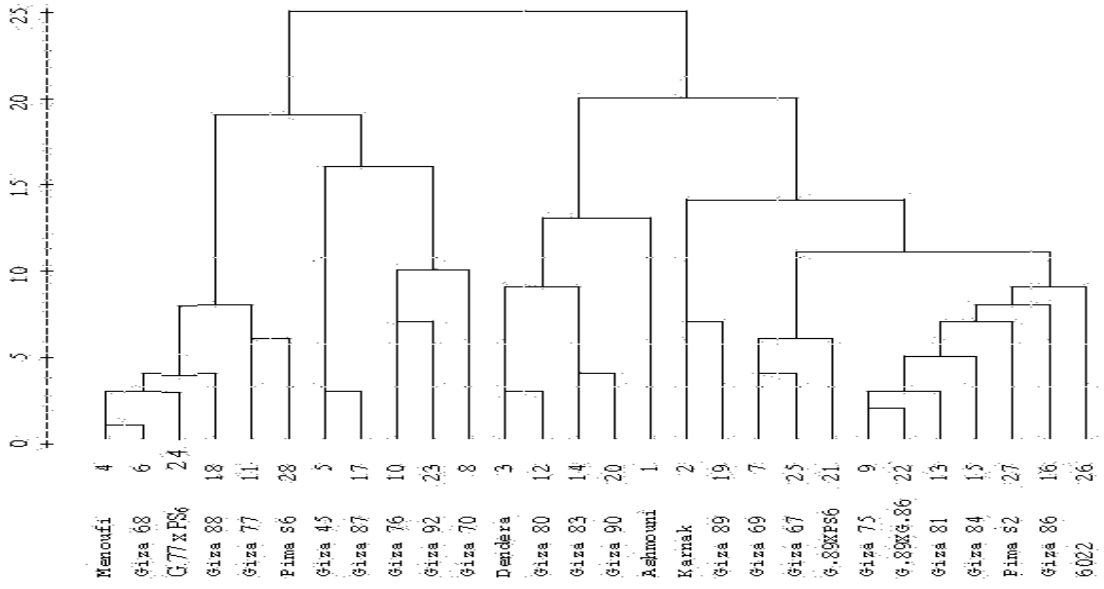

Fig.(2). Results of the hierarchical cluster analysis shown as a dendrogram based on dissimilarity coefficients among 28 cotton genotypes.

Most of the genotypes having similar pedigree and cooperation in a common parent were grouped in single cluster or two closely related clusters. On contrary, the common parent, Ashmouni, tend to be unique group having divergent distance from the other groups , but , appeared to be nearly related with upper Egypt genotypes (G.90, G.80, G.83 and dandra). It cleary demonstrated the impact of selection pressure increasing the genetic divergence.

The intra-cluster distances (Table 6) was maximum in cluster VIII (1.861) followed by cluster II and cluster IX revealing the presence of diverse genotypes in these clusters. The minimum intra-cluster distances were shown by cluster V followed by clusters III and X indicating that the genotypes within these clusters were similar. The genotypes Ashmouni, Giza70 and the Russian variety 6022 formed three unique clusters. Maximum inter cluster distance was observed between cluster III and V followed by cluster I and V, and cluster I and IX indicating maximum diversity between the genotypes of these clusters with respect to the traits considered. High hetrotic response and good segregates could be obtained from the genotypes included in these clusters Cox *et al.,* (1985) and El-Mansy (2005). However, the lowest inter clusters distance were between VII and IX ,also between cluster VI and XI indicating the similarity for most of the characters among the genotypes of the respective cluster.

It is interesting to note that the Egyptian cultivars Menoufi is a common parent of the recent extra long staple varieties, and most of cultivars were developed from mating between closely related parents. On the other side, the best successful cultivars of long staple were developed from mating between closely related parents and Giza67 as a common parent.

The large number of cultivars developed from closely related parents indicated that there are sufficient variability or mechanisms to create variability, to make breeding progress in narrow germplasm base. Unless methods are improved to transfer useful allelic variation from diverse to adapted germplasm without negative agronomic effects, cotton germplasm resources will continue largely and the trend towards increased genetic uniformity.

The cluster mean for each of six characters are presented in Table (7). Cluster V showed the highest cluster mean values for fiber quality characters followed by clusters IX, IV and VII. The genotypes of these clusters were developed from mating of closely related parents. The promising cross (Giza77 x Pima S_6) developed from the mating between two closely related parents Giza77 and Egyptian American variety Pima S_6 and gave values surpassed all genotypes of this category.

The same trend was found in cluster VIII. This cluster consisted of most long staple varieties with Egyptian American variety Pima S_2. The commercial cultivar Giza86 in this cluster developed from mating among two closely related parents, Giza75 and Giza81, and both parents characterized by high yield potential with fiber properties. This cultivar surpassed all Egyptian cultivar for yield potential. Therefore it planted in 75% of cultivated area in Egypt. The two common parents were in cooperation with immediate parent Giza67, also grand parent Giza30 and both parents are closely related.

Table (6): Average inter and intra cluster distances.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	VI
I	0.000	4.747	3.700	5.295	6.872	5.510	4.550	5.706	6.604	2.212	4.991
II		1.668	5.516	2.991	4.692	5.391	2.668	3.980	3.970	3.688	5.439
III			1.051	4.059	7.149	4.676	3.552	3.808	3.624	2.606	3.345
IV				1.264	6.228	5.550	1.803	2.384	1.993	4.172	4.918
V					1.009	3.422	4.776	5.171	5.914	5.930	4.644
VI						1.318	3.873	3.823	4.137	3.376	1.666
VII							0.000	2.767	1.524	2.922	3.384
VIII								1.861	3.702	2.959	3.257
IX									1.503	5.075	3.315
X										1.151	3.913
VI											0.000

Table (7): Cluster mean of the contributed characters in 28 genotypes of cotton.

Clusters	L %	LI	MR	PI	2.5% SL	(+b)
I	36.23	6.48	4.37	8.70	30.90	12.30
II	35.99	6.60	4.30	9.93	32.47	7.85
III	39.12	7.46	4.60	10.40	32.08	12.28
IV	37.29	6.69	3.82	11.06	35.31	11.63
V	34.52	5.91	3.37	11.48	35.82	8.98
VI	38.72	7.06	4.52	10.48	31.72	8.07
VII	38.29	7.14	4.50	10.60	35.43	8.95
VIII	38.84	7.54	4.45	10.46	33.21	8.98
IX	36.81	6.35	3.92	11.27	35.27	8.13
X	37.39	7.13	4.45	9.90	31.93	11.53
VI	37.31	6.49	4.47	10.33	33.47	9.55

It is interesting to note that excluding reselection, most of Egyptian cultivars were developed from mating between two closely related parents and about four of the remaining 28 cultivars had an immediate parent. May *et al.,* (1995) showed that although the average coefficient of parentage among 126 cotton cultivars released between 1980 and 1990 was low (0.07), the coefficient of parents among regionally-adapted cultivars was as high. Thus, the high frequency of closely related parents in a final cross for successful cultivars reflects the fact that new cultivars were for the most part developed from high yielding closely related, locally-adapted cultivars.

In contrast, to the widely held few that a large genetic distance among parents facilitates the development of superior progeny. Thus successful cultivars could be developed from both closely and distantly related parents (Esbroeck and Bowman, 1998).

The weak relationship between parental diversity and cultivar improvement has several probable explanations. There may be sufficient allelic variation, mutation or recombination in the mating of closely related parent to result in improved agronomic performance and/or coefficient of parents may not reflect true genetic distance. There were a number of cultivars developed from reselections indicates as that there were sufficient recombination in mating of closely related parents to improve agronomic performance.

This genetic distance information could be useful in breeding programs in order to introduce important traits as higher genetic distance increases heterosis and selection efficiency .However, more extensive molecular data are needed in order to achieve general conclusion about the relationship between cotton genotypes.

Cotton breeders desire to increase genetic diversity among new cultivars, while at the same time maintaining the complex of desired agronomic and quality traits present in existing commercial varieties. Developing such a combination can be difficult, as the introgression of new genetic material is expected to disturb genetic complex responsible for desirable traits. The use of crosses among divergent cultivars could be a means to achieve both ends. However, more extensive molecular data are needed in order to interpret the best general conclusion about the relationship between cotton genotypes.

Genetic similarity and relationships among genotypes using SSR (microsatellite) markers

Microsatellite markers (SSR) showed considerable genetic variation among twenty eight cotton genotypes (Fig 3) Out of the 52 bands generated from the SSR primer pairs, 44 bands were polymorphic accounting for 84.6% of the total number of generated bands with an average of 7.3 polymorphic bands per primer pair. The total number of bands generated from each primer pair was between five to 13 bands for primer pairs C2-0109 and SSR3, respectively with an average of 8.7 bands per primer pair, while the polymorphic bands percentage ranged from 66.7% for the primer pair M11 to 100% for the primer pair M8. Furthermore, the

Microsatellite marker were able to reveal genetic variation among cotton genotypes. Dongre *et al*., 2007 found that 17 out of the 25 microsatellite markers produced a total of 56 polymorphic bands, four markers were monomorphic and the remaining four produced non-scorable and non-reproducible bands .Furthermore Khan *et al*., 2009 employed thirty-four of the 57 SSR primer pairs displayed polymorphism and 122 (60%) of the 204 SSR bands detected by these polymorphic primer pairs were polymorphic across the cultivars. The number of polymorphic alleles detected per primer pair ranged from one to eight with an average of 3.6 alleles per primer pair.

Determining true genetic dissimilarity between individuals is an important and decisive point for clustering and analyzing diversity within and among populations (Esmail *et al.,* 2008), because different dissimilarity indices may yield conflicting out comes the genetic similarity coefficients to calculate genetic distance among 28 cotton genotypes evaluated using Microsatellite loci ranged from 88.9 to 38.5 (Data not shown). The highest similarity (88.9) was scored between Giza83 and Giza90, and the lowest similarities 38.5 and 41.7 were detected between Giza 90 and Giza 70 and Giza 83 and Giza 84 respectively. This is expected between the genotypes were varied from each other in their background our results are in agreement with findings by Candida *et al.,* (2006) and Sami *et al.,* (2006).

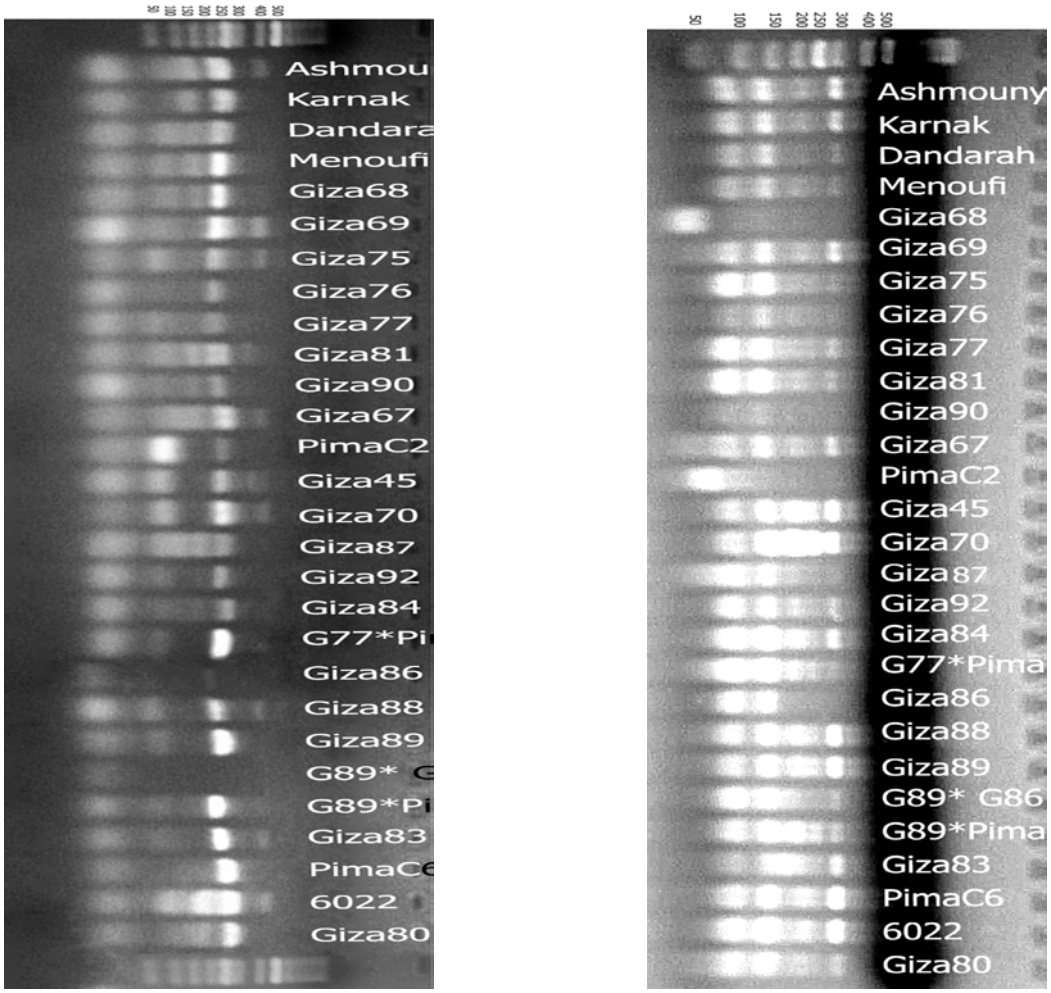

Fig.(3): SSR amplification of 28 cotton germplasm.

Clustering of 28 Cotton genotypes based an SSR markers using UPGMA cluster analysis revealed several variation patterns (Fig 4). First, there were nine major clusters at 20 Euclidean distances about (60%) of similarity. Cluster I consisted of seven genotypes and was further divided into two sub clusters, cluster II contained four genotypes. It appears that clusters IV and III were less similar (or more distinct form) that other seven clusters. Second, clustering was not associated with the periods of cultivar release, as each cluster consists of genotypes from different breeding periods. Third, genotypes were not fully clustered according to their parentage. For example, two cultivar Giza89 and Giza86 shared the same parent Giza75 and were grouped at three different clusters.

Inconsistencies between cultivar clustering and common parents should not be surprised for these cotton genotypes with such broad genetic base (Khan *et al.,* 2009). Also, the limited sampling of the cotton genotypes revealed by SSR primer pairs which used in this study may contribute to such inconsistencies. Application of more mapped markers across the genome would improve the resolution to the genetic relationships of these cotton genotypes (Dongre *et al.,* 2007). However, the estimated genetic relationships still offers a useful guide for cotton breeding as they are more informative than parental selection and traditional pedigree analysis (Bowman *et al.,* 1996).

Data from Figure (4) cleared greater genetic distinctiveness among cotton genotypes as measured by average dissimilarity. This suggests greater distinctiveness of the genetic background for the genotypes. This was clearly among the ancient cultivars rather than modern cultivars. This may reflect the consequence insufficient effort in the introgressions of diverse germplasm into the breeding programs. The relative measure of genetic distinctiveness could provide useful information for selecting specific germplasm with distinct genetic background for a breeding program.

Finally, results from morphological measurements and SSR markers are complementary for each other in studying the genetic divergence and relationships among genotypes and both gave essential information for understanding genetic diversity of Egyptian cotton germplasm. This will provided a useful guide for conserving elite cotton germplasm and developing future cotton breeding programs.

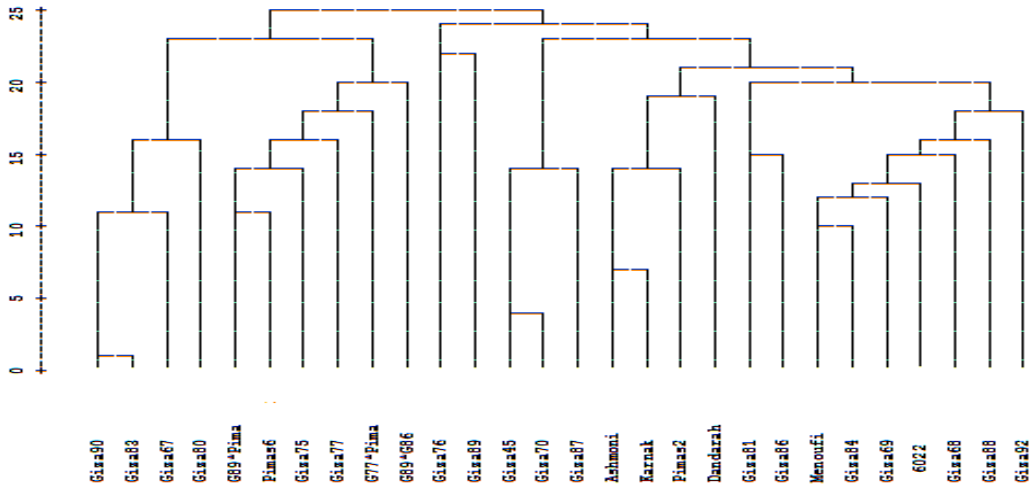

Fig.(4): UPGMA dendrogram constructed based on dissimilarities of 28 cotton germplasm

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التباعد الوراثي بين الأصول الوراثية للقطن وعلاقتها بتطور الأصناف باستخدام الصفات المحصولية وتقنية الـSSR

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الملخص العربي

يعتبر دراسة التباعد الوراثي والعلاقات الوراثية بين التراكيب الوراثية المختلفة من الأهمية لمربى القطن وعلى هذا أجرى هذا البحث بمحطة البحوث الزراعية بسخا وتم استخدام ٢٨ تركيب وراثي للقطن على أساس دراسة صفات معدل الحليج ، ومعامل الشعر وقراءة الميكرونير ، قيمة البرسيلى ، وطول التيلة ، درجة الاصفرار ، وإجراء تحليل الـSSR لهذه التراكيب المختلفة .

وكانت أهم النتائج المتحصل عليها كالتالي :

٩– أظهر تحليل التباين وجود اختلافات معنوية بين التراكيب الوراثية المختلفة لكل الصفات تحت الدراسة .

١٠–أوضح التحليل المتعدد باستخدام تحليل التمايز Discriminant function analysis أن الثلاث عوامل الأولى كانت معنوية وتمثل ٨٠.٤% من التباين الكلى بين التراكيب الوراثية .

١١–كما أوضح التحليل أن العامل الأول يمثل ٦٤.٦% من التباين الكلى وكان أكثر تأثراً بصفات معامل الشعر يتبعها قراءة الميكرونير ، بينما كان العامل الثاني Second function يمثل ١٥.٨% والثالث ١٢.٢% من التباين الكلى .

١٢–تم توزيع التراكيب الوراثية (٢٨تركيب) على أساس الثلاث عوامل الأولى في عشرة مجاميع رئيسية .

١٣–تراوحت قيم Euclidean distance بين ٠.٥ بين الصنفين المنوفى وجيزة ٦٨ إلى ٦٠.٨ بين الصنفين جيزة ٤٥ وجيزة ٨٠ .

١٤–كما تم توزيع التراكيب الوراثية على أساس عدم التشابه النسبى بينها على إحدى عشر تجمع حيث كانت أكبر مسافة داخل التجمع رقم VIII والتجمع رقم II واللذان يحتويان على ٦ ، ٢ تراكيب وراثية على التوالي ، بينما كانا التجمع رقم III والتجمع رقم V أكثر تباعداً فيما بينهما ، وأظهر التجمع رقم VII ، IX علاقة قرابة نسبية.

١٥–أظهرت ٤٤ حزمة من بين ٥٢ حزمة من حزم الـSSR اختلافات بين الأصناف أي ما يقرب من ٨٤.٦% من الحزم الكلية ، بينما أظهر تحليل التشابه على أساس نتائج SSR أن معامل التشابه Similarity cofficient يقع بين ٨٨.٩% إلى ٣٨.٥% .

١٦–تم توزيع الـ٢٨ تركيب وراثى إلى ٩ مجموعات رئيسية على أساس عدم التشابه النسبى لنتائج الـSSR

كما أوضحت النتائج أن القياسات المحصولية واستخدام الـSSR متكاملان لدراسة وإيضاح التباعد الوراثى والعلاقة بين أصول القطن المصرى ، مما يفيد الحفاظ على صفوة السلالات والتراكيب الوراثية الجيدة وتطور برامج التربية المستقبلية .