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MOLECULAR FINGERPRINTING IN IDENTIFYING GENETIC POLYMORPHISM AND RELATIONSHIP WITH QUANTITATIVE TRAITS IN SOME COTTON GENOTYPES

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ABSTRACT

This study aimed to identify the relationship between agronomical traits, yield components and molecular markers among 37 genotypes of Egyptian cotton . Two experiments were done in this work, the first one was concerned to studying morphological traits ,as well as, yield and its component. The second experiment was carried out using different molecular markers to characterize the relationship between polymorphism of ten agronomic traits using different ways of molecular markers based-PCR i.e., RAPD, ISSR and SSR. Many primers gave polymorphic bands which could be considered as a positive or negative markers for agronomical traits and yield components traits based upon the presence or absence of unique band (s) in a specific genotype. The results indicated that the SSR technique was more effective in detecting high level of polymorphism because of highest percentage of polymorphic bands compared with the other molecular markers techniques used herein. This analysis could be a useful tool to genetic distinctiveness among cotton genotypes. This reflects the possibility of utilizing differences between cotton genotypes to improve the economical traits in cotton through the introgressions of diverse germplasm into breeding programs. Hence, the breeder could be used biochemical and molecular markers as rapid and accurate method for identification and facilitate classification of morphological traits and yield components to study germplasm management for genetic improvement of Egyptian cotton.

Key words: Molecular fingerprinting, biochemical molecular markers, morphological traits and yield components ,Egyptian cotton.

INTRODUCTION

Cotton (*Gossypium spp)* is one of the most intensively cultivated species worldwide in more than 80 countries. Cotton constitutes the most important textile plant in the world and is one of the most important crops for the production of oilseed (Zhang *et al.,* 2007). It is also one of the most important crops in Egypt. Molecular genetic markers such as isozymes and markers based-PCR were used to study the genetic variation among different cotton genotypes. While, the mapping of morphological traits continues (Percy and Wendel 1990; Kohel and Bird 2002), the advent of molecular markers (e.g., RFLPs, RAPDs, AFLPs, SSRs, and SNPs) has dramatically altered the utility and application of genetic linkage mapping in cotton. Although isozymes can rightly claim to be the first “molecular” marker, it was not until DNA sequence differences among organisms could be visualized directly that the true era of molecular markers began. The advantage of molecular markers is that, unlike morphological markers, they are only limited by the number of nucleotide differences among individuals. Even a single

nucleotide substitution can be used as a molecular marker. Molecular markers are phenotype-neutral, whereas morphological markers may be difficult to maintain and have deleterious effects on other traits. Linkage analyses with morphological markers and correlations with chromosome position (QTL) helped the development of the framework for mapping molecular markers. With a nearly unlimited pool of genetic markers, cotton geneticists could construct linkage maps of entire genomes that could be used to dissect complex traits (Lin *et al.,* 2005 and Mei *et al.*, 2004) and compare the structure of related genomes (Brubaker *et al.,* 1999; Rong *et al.,* 2004 and Desai *et al.,* 2005).

Molecular markers have been widely used in genetic analyses, breeding studies and investigations of genetic diversity and the relationship between cultivated species and their wild parents (Abou El-Yazied, 2004; Sofalian *et al.,* 2009; Kurt *et al.,* 2011; Mokrani *et al.,* 2012; Abdellatif *et al.,* 2012 and Abdellatif and Soliman 2013).

The present investigation aimed to study genetic variability and polymorphism among cotton genotypes using Markers based-PCR such as RAPD, ISSR and SSR as a biochemical markers to identify the relationship between agronomical traits and molecular markers.

MATERIALS AND METHODS

Thirty-seven Egyptian cotton genotypes (*Gossypium barbadense L*.) were used in this study (Table1).

Table 1: Pedigrees and region of 37 cotton genotypes used in this study.

Number	Name	Origin	Number	Name	Origin	Number	Name	Origin
1	Ashmouni	Egypt	14	Giza 85	Egypt	27	Pima S6	US-Egypt
2	Dandara	Egypt	15	Giza 89	Egypt	28	Pima 62	US-Egypt
3	Menoufi	Egypt	16	Giza 70	Egypt	29	Pima high	US-Egypt
4	Giza 45	Egypt	17	Giza 86	Egypt	30	BBB	Australia
5	Giza 67	Egypt	18	Giza 87	Egypt	31	Suvin	India
6	Giza 68	Egypt	19	Giza 88	Egypt	32	Sea	US-Egypt
7	Giza 75	Egypt	20	Giza 92	Egypt	33	S.I	Sea
8	Giza 71	Egypt	21	Giza 89xGiza 86	Egypt	34	C.B58	US-Egypt
9	Giza 74	Egypt	22	Giza 77xPima S6	Egypt	35	24022A	Australia
10	Giza 77	Egypt	23	G84 (G.70x	Egypt	36	Karshnisky	Russia
11	Giza 80	Egypt	24	10229xGiza 86	Egypt	37	Giza 76	Egypt
12	Giza 81	Egypt	25	Giza 89xPima S6	Egypt			
13	Giza 84	Egypt	26	Giza 75xSea	Egypt			

A field experiment designed to evaluate the performance of agronomical traits in cotton genotypes was conducted at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate during the cotton growing seasons of 2011 and 2012 with randomized complete block design using three replicates. Each plot consisted of one row 4.2 m in length with 70 cm between rows. Hills were spaced by 35 cm apart and comprised of one plant/hill which giveng 12 plants per row.

Ten agronomic traits were recorded from 10 randomly selected plants per replicate. These traits included the position of first fruiting node (P.F.FN), number of fruiting branches per plant. (NO.F.B./P.), days to first flower (D.F.F.), total chlorophyll content (TCC) (mg/ds2, Plant height in centimeter (P.H), boll weight in gram (BW), seed cotton yield/plant in grams (SCY/P), lint cotton yield/plant in grams (L.C.Y/P.), lint percentage (L.P%) and seed index in grams (S.I.).

DNA isolation, purification and quantification: Cotton seeds were grown in the greenhouse for 10 days, and leaves of seedlings were collected and grinded in liquid nitrogen using pestle and mortar. About 0.5 g of the grinded tissue was transferred in 1.5 mL sterilized Eppendorf tube. DNA isolation and purification was carried out using modified cetyl-tetramethyl ammonium bromide (CTAB) method (Dellaporta *et al.* 2007).

RAPD analysis : A total volume of 20 µl PCR was used which is containing 1.0 µl (50ng template DNA), 0.2 µl dNTP,s (10 mM), 1.6 µl Mg Cl2 (25 mM), 2.0 µl 10X buffer (10 mM tris, pH 8.0, 50mM KCl and 50 mM ammonium sulphate), 4.0 µl primer (15 pmole), 0.1 µl taq polymerase (10u/ µl). The volume was brought up to 20 µl by autoclaved double distilled H_2O.

The PCR cycling condition involved initial denaturation at 94°C for 5 min. followed by 35 cycles of amplification under the following by 35 cycles parameters, template denaturation at 94°C for 1 min., primer annealing at 36°C for 1.5 min and primer extension at 72°C for 2 min., final extension at 72°C for 7 min was given, followed by storage at 4°C. PCR thermocycler machines from Biometra (Germany) T-Gradient thermoblock were used.

ISSR analysis: For ISSR analysis, seven primers were selected in order to carry out the ISSR analysis.PCR amplification conditions were as follow: each 25µl PCR reaction solution contained 1.0 µl (50 ng template DNA), 1.0 µl dNTPs (10 mM), 2.5 µl Mg Cl2 (25 mM), 2.0 µl 10X buffer (10 mM tris, pH 8.0, 50mM KCl and 50 mM ammonium sulphate), 2.0 µl of each primer (0.5µM), 0.25 µl Taq polymerase (5u/ µl). The volume was brought up to 25 µl by nuclease-free water. The PCR-ISSR cycling condition involved initial denaturation at 95°C for 2 min followed by 35 cycles of amplification for 35 cycles, template denaturation at 95°C for 1 min (annealing at 48°C for 1 min) and primer extension at 72°C for 2 min, final extension at 72°C for 5 min was given, followed by storage at 4°C agarose (1.5%) was used for resolving the PCR products and 1 kb DNA marker as a standard DNA was used in the present study. The run was performed for 1 hour at 50 volt in SDE-PLAS submarine (10cm x 10cm). Bands were detected on UV- trans-illuminator,

photographed by Gel documentation system and according to analysis by Phoretix program 1D gel analysis software version 4.01.

SSR analysis: Thirty-seven genotypes were subjected to DNA polymorphism screening using five SSR primers to identified from cotton marker database (CMD) (Table 2). PCR amplifications were performed in a 20 µl reaction volume: each reaction contained 1.0 µl template DNA; 0.10µl Taq polymerase, 4µl of 5X buffer, 1µl of 10mM of each of the four (dNTPs), 1.0µl of 10 mM forward and reverse primers. The volume was brought up to 20 µl by autoclaved double distilled H_2O. The amplification protocol of 5 min at 94C°, 35 cycles, was perfrmed with 40 sec at 94°C; 40 sec at lower annealing temperature of the primer about 50 up to 68°C; 1min at 72 °C, and a final extension step of 10 min at 72 °C (Plaschke *et al.,* 1995).

Electrophoretic resolution of PCR product : A 3% agarose gel system was used to resolve the low molecular weight DNA molecules. The gel was prepared by gradually dissolving the agarose powder in 1x TBE buffer to ensure the complete dissolving. After boiling and cooling down (~65^{o}C), diluted EtBr staining solution was added in 2.5µl /100ml. The gel was left for polymerization at room temperature and stored at 4°C for at least 30 min. Samples were loaded after mixing with 1x loading dye and run in 1x TBE buffer at 50 volts for 2.5 h., Gel photos were taken using gel documentation system.

Diversity Analysis: The amplified RAPD , ISSR and SSR DNA bands representing different alleles were scored as different genotypes. For each marker, allelic bands were compared against a 100bp DNA ladder. Then, fragment data was converted into the binary encoded allelic data to apply the multivariate analyses. The total number of bands and the number of polymorphic bands were calculated as well as the polymorphic information content (PIC)

The similarity matrix using Nei and Li (1979) genetic distance for SSR characterization was also used for principal coordinate analysis (PCoA) with the Dcenter, Eigen, Output, and Mxplot subprograms in NTSYS-PC.

Statistical analysis:

Analysis of variance was subjected for all traits from 37 genotypes at two seasons and differences between 37 genotypes were tested for significance according to the regular “F”-test. As outlined by Cochran and Cox (1957). The differences among genotypes mean were determined according to Steel and Torrie (1960). using the value of least significance difference (L.S.D) at 0.001 level of probability .

Software: Quantity one software (Gel Doc, Bio-Rad Laboratory, Inc.) was used to estimate the length of amplification product and in capturing gel images. NTSYS Pc 2.1 was used in cluster analysis and principal coordinate analysis. Microsoft excel worksheets were used to calculate polymorphic information content (PIC) which was calculated according to Anderson *et al.* (1993) using the following simplified formula: $PIC_i = 1- \Sigma p2ij$

where pij is the frequency of the jth allele for marker ith summed across all alleles for the locus. SPSS version 13 was used for principal component analysis. MSTAT version 5 was used for analyzing morphology data.

RESULTS AND DISCUSSION

An analysis of variance of 37 parental varieties through two seasons was done and the obtained results for agronomic traits and yield components are presented in Tables 2 and 3. The mean squares of genotypes were highly significant for all agronomic taits and yield components. The results indicated the presence of significant differences between genotypes for all traits. This finding cleared the presence of large variations among these traits. Therefore, comparison tests between genotypic means could be done. The use of the least significant differences (L.S.D.) method is considered a valid test, since the F-tests were significant.

Table 2: Analysis of variance of 37 genotypes for agronomic traits.

S.O.V	d.f	P.F.F.N		D.F.F		NO.F.B./P.		Chlorophyll ratio		Plant height	
		2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Rep.	٢	٠.٠6	٠.١١	١.٥6	٧.١٢	٠.٩1	١.٥٧	١.٥٥	٠.٠٦	٣٥.٤٥	٣٨.٢٠
Genotype	٣٦	٣.٩**	٢.٨**	٥٤.٦**	٧.٩**	١٢.٦**	١٨.٣**	٨.٦٣**	١٠.٣**	٧٣٣.3**	٤٩٦.٨**
Error	٧٢	٠.07	٠.١1	١.٦٧	٠.٨8	٠.٥٥	٠.٦5	٠.٧٣	٠.٨٧	٤.٦٦	١٠.٣٦

****, Highly significant at 0.001**

Table 3: Analysis of variance of 37 genotypes for yield and yield components traits.

S.O.V	d.f	B.W.		S.C.Y./P.		L.Y./P.		(L. %)		S.I	
		2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Rep.	٢	٠.٠٦	٠.٠٦	٤٤٩٨.0	٤٨١٠.0	٦٩٠.٤	٩٩٦.0	٠.٩٥	١.٩١	٠.١٣	٠.٠١
Genotype	٣٦	٠.١3**	٠.٠9**	٦٠٠٨٣.0**	٢٣٦٠٨.0**	٨١٩٦.١**	٣٤٠١.0**	٦.٠٨**	١٠.٧8**	٠.٥6**	٠.٦٢**
Error	٧٢	٠.٠2	٠.٠3	٤٥٨٣.0	٧٦٠١.0	٦٨٥.٣	١٠٦٤.0	٠.٩٠	١.٢٨	٠.١1	٠.١1

****, Highly significant at 0.001**

The mean values of genotypes for agronomic traits and yield components are presented in Ttable 4 .The results showed that the lowest desirable mean values for the position of the first fruiting node character were obtained from genotype Menoufi followed by Karchenky, Giza75 X Sea and Giza 89XPS_6. However, the most desirable lowest mean value for the date of first flower was obtained from the genotype Karchenky followed by Giza 93, PS_6, Giza 89 and BBB) through the two seasons. On the other hand, the most desirable highest mean values for number of fruiting branches per plant were obtained from genotype Giza 45 followed by Giza76, Menoufi and Giza 87. Also, chlorophyll ratio showed the highest mean values for Ashmouni, Giza 85, BBB, p_{62}, karchenky. Similarly, Giza 87 followed by Dandara and Pima high percentage, Ashmouni and Menoufi appeared highest mean values for plant height in the two seasons. Concerning, the mean values of yield and its component traits for 37 genotypes are listed in Table 4.

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For boll weight (B.W.), the data showed the highest mean values were obtained from 10229 X Giza 86, Giza 89 X Giza 86 and Giza 75XSea. As well as, the seed cotton yield per plant showed the highest mean values from C.B58, Giza 89 and Ashmouni. Regarding the lint yield per plant, clearly showed that the highest mean values were obtained from Giza 84 X (Giza 70XGiza 51B) X P_{62}, $C.B_{58}$ and Giza 89. With regard to the lint percentage the highest mean values were obtained from Pima high percentage, Giza 85 and 10229XGiza 86. Moreover, the genotypes Dandra, 10229 X Giza 86 and Ashmouni showed the highest mean values for seed index in the two seasons.

RAPD marker

Eight random primers were used to identify the genetic variation and screening the DNA of all genotypes in respect to high or low values of each trait. A high level of DNA polymorphism was detected by RAPD technique. For the thirty seven genotypes, RAPD markers amplified using RAPD primer.

The results in Figure 1 and Table 5 showed that, the amplified fragments ranged in size from 85 to 755 bp, with total 158 and 157 polymorphic bands which representing of 99.36% polymorphic with primer1. While, primer 2 obtained bands ranged from 85 bp to 620 bp, with total 152 and 150 polymorphic bands with average level of polymorphism 98.68%. Primer 3 obtained bands ranged from 200 bp to 935 bp, with total 248 at average level of polymorphism 100 %. Regarding primer 4 it was, showed bands ranged from 200 bp to 700 bp, with total 234 and 233 polymorphic bands at average level of polymorphism 99.57%. The band with Mw 550 bp was absent in Giza 45, Giza 76 and Menuofi which recorded the highest mean values for number of fruiting branches per plant and it was present in genotypes Giza 89 X Giza 86, Giza 74 and Giza 86 which recorded the lowest mean values for the same trait, this band can be considered as a negative marker for the number of fruiting branches per plant.

The primer 5 obtained bands ranged from 50 bp to 665 bp, with total 195 bands and 194 polymorphic bands at average level of polymorphism 99.48%. The band with Mw 465 bp was found in genotypes Giza 87, Dandra and P.H.P which recorded the highest mean values for plant height and it was absent in Suven, Giza 89 X Ps_6 and P_{62} which recorded the lowest mean values for the same trait. This band could be considered as a positive marker for plant height. Concerning primer 6 obtained bands, it was ranged from 70 bp to 760 bp, with total 241 bands and 240 polymorphic bands with average level of polymorphism 99.58% . The band with Mw 630 bp could be considered as a positive marker for lint yield per plant because it was found in Giza 84 X (Giza 70 X Giza 51B) X P_{62}, C.B58 and Giza 89 which recorded the highest mean values for lint cotton yield per plant, while it was absent in genotypes Giza 77, Dandra and BBB which recorded the lowest mean values for the same trait.

For seed index the primer 6 showed positive and negative marker,while the band with Mw 630 bp was present in genotypes Dandra, 10229XGiza 86 and Ashmouni which recorded the highest mean values for seed index and it was absent in Giza 76, Giza 87 and Giza 70 which recorded the lowest mean values for the same trait.This band could be considered as a positive marker for seed index. On the other hand, the band with Mw 485 bp was present in genotypes which recorded the lowest mean values and was absent in genotypes which recorded highest mean values for seed index, this band can be considered as a negative marker for seed index. The band with Mw 295 bp could be considered as a positive marker for chlorophyll content, this band was found in genotypes which recorded the highest mean values for chlorophyll content such as Ashmouni, Giza 85 and BBB and it was absent in genotypes recorded the lowest mean values for the same traitsuch as Giza 70, Giza 76 and Giza 87.

Concerning primer 7 obtained bands which ranged from 65 bp to 295 bp, with total bands 121 at average level of polymorphism 100 %. Meanwhile, primer 8 showed bands ranged from 65 bp to 405 bp, with total 108 and 106 polymorphic bands at average level of polymorphism 98.15%. This primer showed a band having 295 Mw found in Giza 87, Dandra and P.H.P which recorded the highest mean values for plant height and it was absent in genotypes Suven, G.89XPs6 and P62 which recorded the lowest mean values for the same trait. Also, the band with Mw 230 bp was present in Giza 86, Ashmouni and Giza 76 which recorded the highest mean values for days to first flower (D.F.F.) trait and was not found in genotypes Karchenky, Giza 93 and Ps_6 which recorded the lowest mean values for the same trait. This band can be considered as a negative marker for this trait. On the another hand, the band with Mw 245 bp was present in genotypes Karchenky, Giza 93 and Ps_6 which recorded the lowest mean values for days to first flower and it was absent in Giza 86, Ashmouni and Giza 76 which recorded the highest mean values for days to first flower , this band could be considered as a positive marker for days of first flower.

It can be concluded that RAPD markers were found to reveal sufficient genetic diversity and a high level of polymorphism. Low levels of correlation were existed between agronomical and RAPD based genetic similarities obtained in the this study. RAPD analysis reflected the true expression of genotypes, while agronomical analysis encompassed the expression of genotype, environmental effect and their interactions. Agronomical traits are not consistent, whereas RAPD analysis appeared to provide more accurate estimates and utility of genetic diversity measurements.

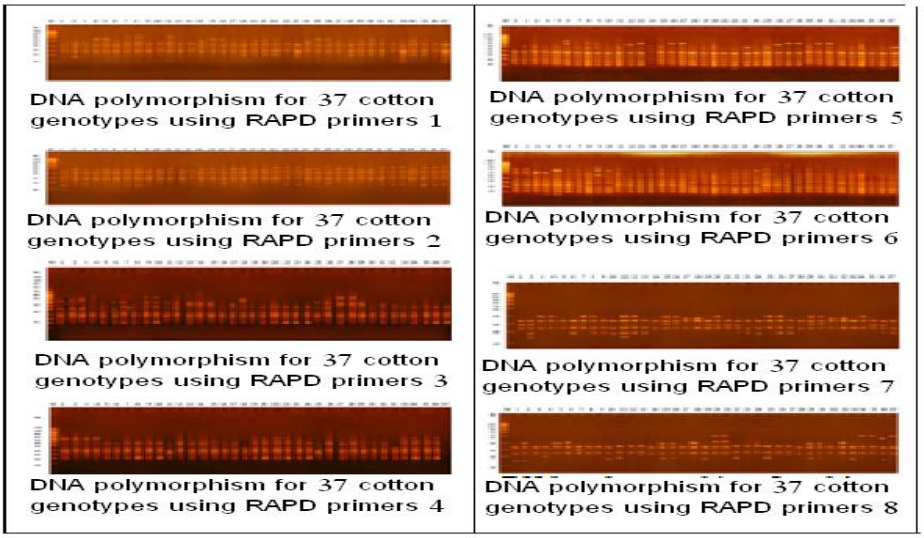

Figure1: lectrophoresis of PCR products patterns for 37 cotton genotypes using eight RAPD primers .

All methods have an advantages and disadvantages for practical applications under different circumstances. Consequently, both methods should continue rendering valuable services to farmers, breeders and genetic resource curators. The overall findings from this study indicated that RAPD analysis of qualitative and quantitative traits, sufficiently detected genetic diversity to differentiate Egyptian cotton varieties. Genetically distinct varieties were identified that could be potentially important sources of germplasm for cotton improvement. Although all methods did not provide exactly the same description of relationships between varieties, they existed some consistency in discriminating varieties which were closely related and ones which were distantly related. RAPDs analysis are more efficient and provide exciting insights (Lu and Myers, 2002; Khan *et al.,* 2009). Application of DNA markers could accelerate the process of finding markers related to specific agronomical traits of interest, such as disease and pest tolerance (Spielmeyer *et al.,* 1998). *Gossypium barbadense* has limited genetic diversity, therefore RAPD analysis may offer a powerful tool for analyzing the inheritance and relationships of important traits in cotton breeding. Therefore, future research should be focused on comparing the two methods in terms of feasibility, efficiency and accuracy by involving more tests over different environmental trials and years (for agronomic traits). Molecular analysis using more primer combinations and different molecular markers, along with costs and benefits, should be included.

ISSR marker

Regarding ISSR – PCR amplification using seven primers appeared high level of DNA polymorphism as detected by ISSR technique. For the thirty seven genotypes, ISSR markers amplified using primer 1 which presented in Figure 2 and Table 5 giving many bands ranged from 1151 bp to 112 bp, with total 118 and 116 polymorphic bands at average level of polymorphism 98.30%, one band with Mw 255 bp was present in Giza 76, Giza 87 and Giza 70 which recorded the lowest mean values for seed index. This band was absent in Dandara, 10229 X Giza 86 and Ashmouni genotypes which recorded the highest mean values for the same trait. This band could be considered as a negative marker for seed index.

Primer 2 showed positive and negative marker for height of first fruiting node.The band with Mw 605 bp could be considered as a positive marker for height of first fruiting node, while the band with Mw 545bp could be considered as a negative marker of height of first fruiting node. Primer3 band has Mw 155 bp which could be considered as a positive marker for days to first true leaf .

Table 5: Primers and their polymorphic bands appeared by RAPD, ISSR and SSR markers.

Primer name	Sequence	No.of bands	Polymorphic bands	% polymorphic	PIC
RAPD primers					
OPA-5	AGG GGT CTT G	158	157	99.36	0.6969
OPA-1	CAG GCC CTT C	152	150	98.68	0.8197
OPA-7	GAA ACG GGT G	248	248	100	0.7482
OPA-8	GTG ACG TAG G	234	233	99.57	0.8003
OPA-9	GGG TAA CGC C	195	194	99.48	0.8372
OPA-10	CTG CTG GGA C	241	240	99.58	0.8694
OPA-11	GTA GAC CCG T	121	121	100	0.7266
OPA-01	TTC GAG CCA G	108	106	98.15	0.7615
ISSR primers					
UBC 848	(CA) 8R*G	118	116	98.30	0.8233
HB12	(CAG) 3GC	124	120	96.77	0.8032
844A	(CT) 8AC	168	163	97.02	0.7937
17889A	(CA)6AC	131	127	96.94	0.8286
UBC 836	(AG) 8Y*A	128	126	98.43	0.7807
UBC 842	(GA)8Y*G	154	152	98.70	0.7661
HB15	(GTG) 3GC	170	169	99.41	0.8143
SSR primers					
BNL1440B	F:CCGAAATATACTTGTCATCTAAACG R: CCCCCGGACTAATTTTTCAA	67	67	100	0.7097
BNL3408A	F: ATCCAAACCATTGCACCACT R: GTGTACGTTGAGAAGTCATCTGC	37	36	98.29	0. 512
BNL3408B	F: AGCAAAATCGAAATTGCAGC R: GGGGGGGATTAGATCCTTTT	74	73	98.64	0.5826
BNL2634A	F: AACAACATTGAAAGTCGGGG R: CCCAGCTGCTTATTGGTTTC	64	64	100	0.7561

Regarding primer4,the band with Mw 500 bp it could be considered as a negative marker for seed cotton yield per plant and lint yield per plant. Concerning primer 6 the obtained bands ranged from 1075 bp to 85 bp, with total 154 bands. The results appeared that one band with Mw 355 bp was

found in Giza 87, Dandra and P.H.P which recorded the highest mean values for plant height and was absent in Suven, Giza 89 X Ps_6 and $P_{62.}$ This band can be considered as a positive marker for this trait. In addition, the band has Mw 405 was found in Giza 76, Giza 87 and Giza 70 which recorded the lowest mean values for seed index and was it absent in Dandara, 10229XG.86 and Ashmouni which recorded the highest mean values for the same trait. This band could be considered as a negative marker for seed index.

Concerning primer 7, obtained bands ranged from 1110 bp to 80 bp, with total 170 bands and displayed high level of polymorphism (99.41%). The band with Mw 595 was present in Giza 85, Giza 89 and Giza 76 which recorded the lowest mean values for boll weight (B.W.) and it was absent in 10229XGiza 86, Giza 89 X Giza 86 and Giza 75 X Sea which recorded the highest mean values for the same trait. This band could be considered as a negative marker for boll weight. In addition, the bands with Mw 555 bp and 160 bp were found in genotypes Dandara, 10229XG.86 and Ashmouni which recorded the highest mean values for seed index and was absent in Giza 76, Giza 87 and Giza 70 which recorded the lowest mean values for the same trait. These bands can be considered as a positive markers for seed index.

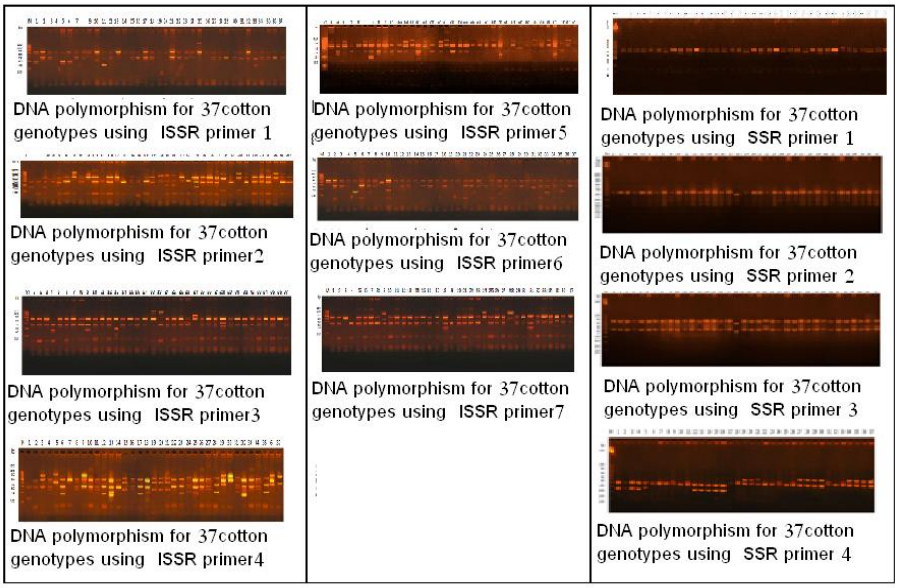

Figure2: Electrophoresis of PCR products patterns for 37 cotton genotypes using ISSR and SSR primers .

SSR marker

A high level of DNA polymorphism was detected by SSR technique which giving the highest percentage of polymorphic bands (98.29 - 100 %) compared with the other molecular markers used in this study. The SSR

markers amplified used primer 1 which presented in Figure 2 and Table 5 gave four alleles ranged from 755 bp to 565 bp, with total 67 bands. The allele has Mw 725 bp was present in Giza 45, Giza 76 and Menoufi which recorded the highest mean values for number of fruiting branches per plant and it was absent in Giza 89 X Giza 86, Giza 74 and Giza 86 which recorded the lowest mean values for the same trait. This allele could be considered as a positive marker for this trait. On the other hand, the allele with Mw 685 was present in Giza 89 X Giza 86, Giza 74 and Giza 86 which recorded the lowest mean values for number fruiting branches per plant (NO.F.B./P.), while it was absent in Giza 45, Giza76 and Menoufi which recorded the highest mean values for the same trait. This allele could be considered as a negative marker for the number of fruiting branches per plant.

Concerning primer 2 it was showed two alleles with Mw405 and 330 bp with total of 37 bands and average level of polymorphism 98.29 %. The first allele (405 bp) was found in all genotypes, while Giza 87 showed the other allele (330bp). With regard to primer 3 the total amplified bands were 74. Six alleles were amplified after using this primer and ranged from 750bp to 330bp. There is no any allele out of the obtained six alleles could be considered related to any studied traits.

Primer 4 showed that 64 bands were amplified with average level of polymorphism 100 % and ranged from 260bp to 45bp. The band with Mw 115bp was present in genotypes P.HP,G.85 and 10229 XGiza 86 which recorded the highest mean values for lint percentage (L%), and it was not found in Giza 87, Giza 92 and Giza 88 which recorded the lowest mean values for the same trait. This band could be considered as a positive marker for lint percentage. On the other hand, the band with Mw 165bp was present in genotypes Giza 87, Giza 92 and Giza 88 which recorded the lowest mean values for lint percentage and it was absent in genotypes P.HP, Giza 85 and 10229 XGiza 86, which recorded the highest mean values for the same trait, this band can be considered as a negative marker for lint percentage. The PIC of the SSR primers ranged from 0. 51 for primer 2 to 0.75 for primer 4.

Thus, the SSR technique is more effective in detecting high level of polymorphism, which is in concurrence with earlier reports in many plant species (Saini *et al.,* 2004; Medini *et al*., 2005; and Maras *et al.,* 2008). The distinctive value of MI for SSR data is related to the effective multiplex ratio. In other words, it depends more on the high number of polymorphic bands obtained per experiment than on the allelic heterozygosity found among accessions. The average number of the polymorphic and the total number of bands per primer pair was higher than the results obtained by Kalivas *et al.,* (2011), who analyzed 29 cultivars of *Gossypium hirsutum* and an interspecific hybrid (*G. hirsutum* x *G. barbadense*) using 12 pairs of SSR markers. They observed that two to four different alleles were amplified at each genomic locus, with a mean of 2.53 alleles per locus. Furthermore, 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. Moreover, Bertini *et*

al., (2006) characterized 53 cultivars using 31 pairs of SSR primers; they obtained a total of 66 alleles with an average of 2.13 alleles per SSR locus. Similarly, Gutierrez *et al.,* (2002) used 60 pairs of polymorphic primers amplifying 69 loci which resulted in 139 alleles with an average of two alleles per locus. However, Liu *et al.,* (2000) used 56 polymorphic primer pairs to amplify 62 cotton loci and produced a total of 325 alleles with an average of 5 alleles per locus. Khan *et al.,* (2009) employed 34 of 57 SSR primer pairs screened that 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. Buteler *et al.,* (1999) claimed that the multi-locus amplification of the SSR is common in species with allopolyploid origin.

Cluster analysis

According to the cluster analysis of all molecular data combined, all 37 genotypes showed that the genetic distance for each genotype combination ranged from 0.67 to 0.80 and were separated into two major clusters using all the data generated from three different molecular markers: RAPD, ISSR and SSR (Fig. 3). Studied genotypes formed two main clusters A and B, the first main cluster was located at the uppermost part of dendrogram and included the old cotton varieties (Ashmouni, Dandara, Menoufi ,Giza 67 and Giza 45) which separated at genetic similarity of 0.71 and created two sub clusters A1 and A2. The second sub cluster included an individual cultivars Giza 45 at genetic similarity of 0.71, this variety was unique in all fiber quality traits and it was considered one of the best fineness cotton variety in the world. Hence it located in separate clustered in the dendrogram. The second main cluster separated at genetic similarity of 0.68 and created two sub clusters B1 and B2. The first sub cluster B1 divided into B11 and B12 at genetic similarity 0.685, while B2 divided into B21 and B22 at genetic similarity 0.69 and include the other remaining genotypes used in this study.

The possibility of utilizing the differences between cotton genotypes was impotant to improve the economical traits in cotton through the introgressions of diverse germplasm into the breeding programs. It was indicated that breeders have not been working in isolation, and the breeding material for the development of these cultivars, but has been shared between the breeding stations and cotton improvement programs.

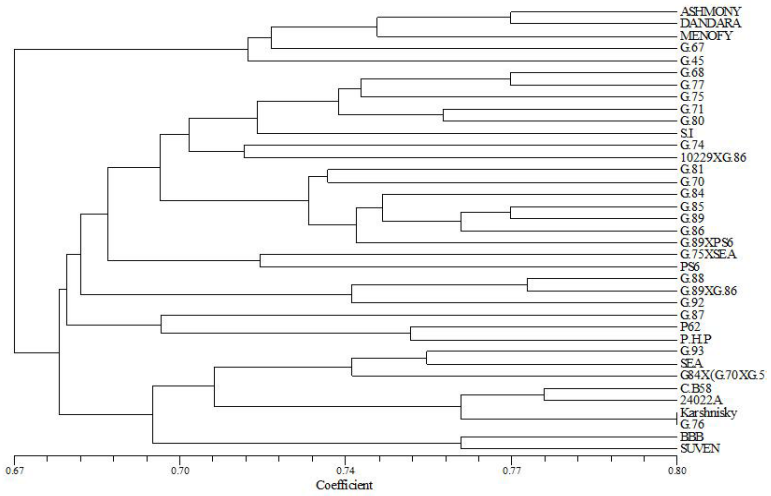

Figure 3:Dendrogram of cotton genotype based on the cluster nalysis of Nei and Li's genetic distance (1979).

Finally, it can be concluded that biochemical and molecular markers were used as rapid and accurate methods for identification of cotton genotypes to facilitate classification of morphological traits and yield components via studying germplasm management to genetically improvement the Egyptian cotton breeding programs.

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البصمه الجزيئية في تحديد تعدد الانماط الجينية وعلاقتها بالصفات الكمية في بعض التراكيب الوراثية من القطن

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يهدف هذا البحث الى تحديد العلاقة بين الصفات المورفولوجيه والمحصولية والمعلمات الجزيئية. وقد اجريت هذه الدراسة على مرحلتين ،المرحلة الأولى وفيها تمت دراسة ٣٧ تركيب وراثى من الناحيه المورفولوجية و من حيث المحصول ومكوناتة . اما المرحلة الثانية وفيها تم إستخدام المعلمات الجزيئية المختلفة لتوصيف العلاقة بين تعدد الانماط المظهرية و بعض الصفات الكمية باستخدام طرق مختلفة من المعلمات الجزيئيه مثل RAPD ،ISSR ،SSR .لتحديد العلاقة بين الصفات المورفولوجية و المحصوليه و المعلمات الجزيئية تم الكشف عن مستوى عال من تعدد الأشكال للحامض النووي DNA عن طريق تقنية RAPD ، ISSR ، SSR للسبعة و ثلاثون تركيب وراثى. تم تحديد عدد من المعلمات الجزيئية الموجبة والسالبة و التى ترتبط إرتباطا وثيقا بالصفات تحت الدراسة على أساس وجود او غياب حزم جزيئيه مميزة (فريده) فى تركيب وراثى معين. وقد اوضحت النتائج ان طريقة الـ SSR كانت اكثر كفاءة فى ملاحظه المستوى العالى من تعدد الاشكال حيث أظهرت أعلى نسبه من الحزم المتعددة الاشكال بالمقارنه بطرق المعلمات الجزيئيه الأخرى المستخدمه فى هذه الدراسه.

وهذا يعكس امكانيه الاستفادة من الاختلافات بين التراكيب الوراثيه فى تحسين الصفات الاقتصاديه فى القطن من خلال ادخال هذة التراكيب الوراثيه المختلفه فى برامج تربية القطن. ومن ثم يمكن استخدام الطرق البيوكيماويه والمعلمات الجزيئيه كطرق سريعه ودقيقه للتعرف وتسهيل توصيف الصفات المورفولوجيه والمحصولية فى التراكيب الوراثيه المستخدمة فى برامج تحسين القطن المصرى.

Table 4 : Mean values of 37 genotypes for agronomic traits and yield components.

No	P.F.F.N		D.F.F		NO.F.B./P.		Chlo. ratio		Plant height		B.W. (gm)		S.C.Y./P.		L.Y./P.		(L. %)		S.I	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
1	7.63	8.17	85.33	68.98	17.67	22.00	51.38	51.20	180.33	178.00	2.95	2.79	800.42	430.93	300.77	153.57	37.55	35.67	10.4	10.90
2	6.70	5.83	84.83	64.52	20.83	25.67	46.63	49.60	182.00	177.6	2.88	2.71	272.17	394.37	101.83	137.57	37.42	34.89	11.1	10.93
3	6.10	5.50	81.08	65.33	22.83	25.50	49.08	47.80	181.83	175.6	2.90	2.81	604.13	409.40	229.98	146.67	38.07	35.80	10.07	10.23
4	7.90	8.50	77.28	67.02	25.83	29.00	47.42	45.20	183.50	167.83	2.87	2.83	531.47	470.70	196.42	161.30	36.98	34.28	9.73	9.80
5	7.80	6.73	73.17	65.00	21.50	22.50	49.45	47.67	176.83	167.67	2.80	2.75	565.28	373.87	195.60	139.87	34.60	37.39	10.03	10.17
6	9.23	8.33	82.02	68.40	22.17	23.83	49.47	47.58	169.11	160.33	3.01	2.73	696.03	482.27	247.95	167.67	35.62	34.67	10.33	10.30
7	5.75	7.50	74.47	68.01	21.83	21.33	49.18	46.65	178.33	171.00	3.16	2.97	587.42	310.73	223.15	120.20	37.75	38.54	9.87	10.37
8	8.65	7.33	82.63	68.33	20.33	22.67	45.07	46.12	176.50	164.33	2.91	2.81	433.00	357.53	162.80	135.90	37.59	38.05	9.67	9.80
9	8.60	8.83	79.42	68.42	18.83	17.83	47.25	45.98	159.67	159.67	2.95	3.11	467.72	468.57	178.67	177.43	38.19	38.02	10.13	10.30
10	8.07	7.00	80.03	65.48	21.33	22.83	48.85	48.15	168.00	175.67	2.78	2.85	285.30	261.53	108.60	98.00	38.20	37.42	10.07	10.07
11	7.25	7.17	83.54	65.78	19.83	21.00	48.57	48.60	146.00	146.00	3.13	2.79	412.23	557.50	158.33	211.87	38.36	37.95	10.23	10.27
12	8.33	7.67	78.50	68.56	21.50	20.67	47.83	46.03	146.83	150.00	3.02	2.82	612.50	428.43	238.63	160.40	38.93	37.45	10.43	10.50
13	8.33	9.00	77.07	68.50	21.83	19.61	46.58	45.80	163.83	172.00	2.98	2.76	633.65	511.00	240.92	182.43	38.00	35.66	9.77	10.17
14	7.37	6.83	78.54	66.92	22.50	24.00	50.28	49.73	168.00	156.33	2.70	2.62	389.83	473.73	151.10	183.93	38.70	38.81	10.37	10.77
15	6.10	6.67	71.66	66.38	24.00	23.50	48.33	44.12	164.83	153.33	2.80	2.65	784.27	452.23	295.07	173.17	37.62	38.30	10.17	10.10
16	8.27	8.17	77.61	68.79	24.00	22.67	44.60	43.25	162.83	159.33	3.08	2.76	489.08	387.00	182.50	141.93	37.28	36.67	9.23	9.50
17	9.53	9.00	82.63	71.83	19.17	19.00	46.07	45.50	179.50	161.67	3.34	3.11	588.68	380.27	216.10	140.47	36.64	36.94	10.43	10.03
18	9.30	8.04	80.77	69.91	24.00	24.17	45.03	43.55	185.83	174.00	3.05	2.85	635.43	328.13	204.15	103.13	31.88	31.26	9.37	9.37
19	9.15	9.17	79.50	67.58	23.33	24.00	47.65	47.37	165.67	168.33	3.11	2.91	431.75	567.63	155.60	188.73	36.05	33.13	9.47	9.53
20	7.77	7.33	75.17	67.05	24.17	21.33	48.15	49.05	177.83	151.33	3.33	3.11	692.85	524.90	240.70	170.33	34.81	32.40	9.93	9.63
21	8.17	6.67	80.18	67.74	17.00	19.33	48.50	48.12	152.00	150.67	3.40	3.12	669.22	527.07	256.62	201.20	38.34	38.12	9.50	9.63
22	8.00	8.50	71.07	66.04	21.33	19.17	46.22	47.72	174.83	168.33	2.99	2.94	442.23	435.10	160.62	158.13	36.32	36.40	9.83	10.03
23	8.47	7.83	77.38	66.56	23.00	19.31	48.50	47.87	162.00	157.00	3.30	3.01	593.73	629.20	227.47	244.20	38.40	38.78	9.77	9.77
24	6.35	6.83	72.83	67.46	20.50	19.33	48.07	48.50	169.83	167.67	3.40	3.30	605.97	524.87	233.93	203.67	38.56	38.80	10.73	11.13
25	6.30	5.88	80.90	64.52	19.83	19.33	48.57	48.65	134.33	137.00	3.00	2.91	415.25	419.37	157.30	157.23	37.86	37.42	9.50	9.93
26	5.80	6.33	74.03	66.17	20.17	19.33	47.80	48.32	168.00	157.00	3.33	3.15	661.77	454.07	238.33	169.03	36.09	37.20	10.40	10.23
27	5.68	6.87	71.53	65.98	23.50	19.33	48.50	48.35	158.17	150.67	3.27	2.94	408.83	403.73	154.53	151.53	37.76	37.51	9.73	10.13
28	6.88	6.83	72.34	66.10	19.33	19.50	49.68	49.08	145.33	139.00	3.02	3.03	314.23	518.80	113.45	176.33	36.19	34.32	10.10	10.27
29	8.17	7.83	79.74	68.57	24.17	22.83	44.67	46.32	182.83	176.00	3.27	3.01	397.47	496.10	154.23	192.30	38.88	38.79	10.23	10.00
30	6.48	6.13	73.30	64.79	20.33	20.17	48.58	50.20	147.33	147.33	2.71	2.86	266.25	490.33	100.55	169.40	37.77	34.59	10.40	10.60
31	6.17	7.17	74.21	65.37	22.33	18.17	48.17	48.42	114.43	116.67	3.07	3.11	580.98	555.63	209.03	191.50	36.13	34.53	10.27	10.40
32	7.60	6.84	79.27	65.97	19.50	20.50	47.35	49.32	158.33	154.67	2.86	2.75	459.32	582.23	167.73	211.37	36.47	36.29	9.73	9.67
33	6.35	6.83	81.83	65.63	21.00	19.33	46.47	47.90	175.33	159.33	3.23	3.13	489.32	502.27	178.53	177.70	36.49	35.43	10.47	10.77
34	8.20	6.50	80.17	67.67	20.67	21.17	45.38	46.03	178.67	165.67	3.33	3.03	704.92	567.70	259.62	211.17	36.79	37.12	9.57	9.57
35	8.63	7.63	84.00	67.21	21.83	20.83	47.13	46.80	153.83	153.00	3.23	2.99	532.27	689.47	199.40	257.40	37.46	37.31	9.40	9.50
36	5.63	6.00	70.73	66.25	20.17	18.50	48.98	48.85	149.67	154.33	2.85	3.00	672.45	451.80	261.65	173.03	38.91	38.31	10.30	10.23
37	8.08	8.50	83.27	68.62	25.50	23.50	44.05	44.52	173.17	163.33	2.87	2.59	365.95	450.70	134.03	165.70	36.63	36.76	9.40	9.33
Mean	7.53	7.35	78.16	67.07	21.56	21.43	47.66	47.40	165.28	159.67	3.05	2.91	526.85	466.73	195.56	170.42	37.17	36.51	10.01	10.11
LSD	0.44	0.53	2.10	1.52	1.21	1.31	1.39	1.52	3.52	5.24	0.2116	0.2625	110.19	141.90	42.61	53.08	1.5458	1.843	0.5360	0.5346