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GENETIC BEHAVIOUR OF SOME EGYPTIAN COTTON VARITIES (*Gossypium barbadense* L.) UNDER STRESSCONDITIONS OF WADI SUDR,SOUTH SINAI

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ABSTRACT

Two successive experiments were carried out at Ras Sudr Agricultural Research Station of DRC during 2000 and 2001 growth seasons to investigate the mean performance (adaptability) of six Egyptian cotton varieties to saline conditions of Wadi Sudr. Also to evaluate the genetic variation and genetic relationships between varieties using random amplified polymorphic DNA (RAPD) markers. Long staple varieties Giza 90 then Giza 83 followed by Dendera were more adapted to saline conditions and attained the significant highest values of yield and its attributes. Meanwhile extra long staple ones Giza 77 as well as Giza 45 were less adapted and exhibited a significant lower values. Five random 10-mer primers were used to amplify DNA via polymerase chain reaction (PCR) and 55 RAPDs were generated. The five primers detected polymorphism in all six cotton varieties. Out of the total amplified bands 60 % were polymorphic. Similarity matrix was generated from the RAPD results as compared by using Nei and Li's (1979) coefficient. Giza 77 was the least similar one with other studied varieties whereas Giza 90, Giza 83 were the greatest similar varieties. Dendrogram generated, by UPGMA analysis, detected that the more adapted varieties to saline conditions, long staple varieties Giza 90, Giza 83 and Dendera grouped together; Giza 45 the extra long staple variety which and Giza 85 the long staple variety showed that significant lower yield and its attributes grouped together whereas Giza 77 extra long staple variety and lowest adapted or significant lowest yielding ability did not cluster with any other variety tested.

The results obtained could be used for selection the suitable parents for breeding program for improving fiber qualities and its adaptability under saline conditions.

Keywords: Cotton *(Gossypium Barbadense* L.) RAPD – Genetic similarity – Genetic *diversity – Yield and its attributes – Saline conditions.*

INTRODUCTION

Cotton, the most important fiber crop in the world, represents one of the major cash crops in Egypt (Abdel-tawab *et al.*, 2001). It is considered as salt tolerant plants, but the degree of salt tolerance varied among different varieties (Maas and Hoffman, 1977). Many investigators (Rathert, 1983; Abdel rehim, 1989; Jafri and Ahmed, 1994; Munir *et al.*, 1995 and Afiah and Ghoneium, 1999) concluded that number of bolls, boll weight, seed index, seed cotton yield, lint/boll and fiber length & strength were decreased with increasing salinity levels and the reduction varied according to salinity level, type of soil and irrigation intervales.

As a consequence of rapid increase of human population in Egypt, the agricultural extension through desert and using a breeding program for improving adaptability (producing elite high yielding cultivars) under desert environment are consider a vital solutions for increasing crop production. The fact that, adaptability is determined both by major and minor genes and that more or less complex co-adapted gene complexes may develop which influence adaptability to specific environments (Allard, 1992) has important consequences for identification of suitable parental lines for hybridization program as well as for breeding and selection methods (Hawtin *et al.*, 1996).

In addition elite adapted genotypes is the most important sources of genetic variation that must be available in breeding stocks in order to make further improvement in crop production under stress conditions (Vetelainen *et al.*, 1996). Estimation of the extent of variation within and between varieties of a species is useful for analyzing the genetic structure of crop gerplasms (Hayward and Breese, 1993), and predicting potential genetic gain in a breeding program (Morene-Gonzalez and Cubero, 1993). A large number of polymorphic markers are required to measure genetic variation (diversity) in a reliable manner. This limits the use of agronomical characters, which are few, lack adequate levels of polymorphism, polygenic and influenced by environmental conditions (Kongkiatn-gam *et al.*, 1995). Molecular genetic markers have developed into powerful tools to analyze genetic diversity (Tatineni *et al.*, 1996). Random amplified polymorphic DNA (RAPD) analysis can reliably determine genetic diversity within a diverse array of *Gossypium germplasm* and various plant species (Multani and Lyon, 1995; Tatineni *et al.*, 1996 and Iqpal *et al.* 1997).

Hence, this study was carried out to investigate the more or less adapted cotton varieties to saline conditions of Wadi Sudr through determine the mean performance of cotton yield and its main attributes for elite six commercial varieties. Also, assess the genetic variation and genetic relationships between more and less adapted studied varieties using RAPD markers. This may be help its great exeunt, in selection of suitble parents for breeding program for improving adaptation of cotton plant under saline conditions of Wadi Sudr.

MATERIALS AND METHODS

Materials:

Seeds of six Egyptian cotton (*Gossypium barbadense* L.) varieties were used in this study (Table 1) and obtained from cotton Research Inst. Agricultural Research Center, Egypt.

Table (1): Names, parentage and characteristics of six Egyptian cotton (*Gossypium barbadense* L.) varieties salinity levels

Number	Name	Parentage	Characteristics
1	Giza 45	Giza 28 (Sakha 3 × Sakha 4) × Giza 7	Extra long stable
2	Giza 77	Giza70 (Giza 59 (Giza36 × Giza 44) × Giza 51 (Giza36 × Giza 40) × Giza 68 (Giza 36(Giza12 × Sakha 3) × Giza 56 (Giza39 × Giza 36)	long stable
3	Giza 83	Giza 67 (Giza 53 (Giza7 × Giza 38) × Giza 30 (Giza 7 × Sakha 11) × Giza 72 (Giza 61 (Giza 39 × Giza 30) × Giza 47 (Ashmouny))	long stable
4	Giza 85	Giza 67 (Giza 53 (Giza 7 × Giza 38) × Giza 30 (Giza 7 × Sakha 11) × Giza 58 (Giza 28 (Sakha 3 × Sakha 4) × Giza 36 (Giza 12 × Sakha 3))	long stable
5	Giza 90	Giza 83 × Dendera	long stable
6	Dendera	Gia 31 (Giza 3)	long stable

Methods:

Main experiments:

Two successive field experiments were conducted under calcareous soil of Ras Sudr Agricultural Experiment Station of Desert Research Center during 2000 and 2001 growth seasons. The six varieties were grown under saline soil (EC 8124 ppm) and irrigated with about 7568 ppm under ground water during the growth season. Each experiment was laid out in randomized complete block design with three replicates. The plot area was 12 m^2 (3x4 m) containing 6 ridges, each ridge was of 4 m length and 50 cm width. Seeds were sown in hills of 25 cm apart and two seedlings were kept in each hill. Seeds were planted on March 28 and April 5, 2000 and 2001, respectively. After 30, 60 and 90 days from sowing N and K fertilizers were added in the rate of 60 kg / fed. (divided into three equal doses) while superphosphate (P_2O_5) was added before sowing at the rate of 30 kg / fed. the other culture practices were properly carried out.

Yield and yield characters studied:

Data were recorded randomly on ten random individual guarded plants from the middle four ridges in each plot for number of open bolls / plant, boll weight (g), lint yield / plant (g) and seed cotton yield / plant (g).

Polymerase chain reaction (PCR) technique and RAPD analysis:

DNA extraction

Genomic DNA was extracted from fresh young leaves of randomly chosen ten plants for each variety, according to the method of Dellaparta *et al.* (1983). All leaves of ten plants from a signle experiment were bulked prior to extraction. DNA was measured by spectrophotometer wave length and gel electrophoresis.

DNA amplification:

Oligonucleotide primers (10-mers) were obtained from Operon Technologies (Alamada, Calif.) according to Williams et al. (1990) with the following sequence:

Primer code	Primer sequence 5\...............3\
B8	GTCCACACGG
B_{16}	TTTGCCCGGA
Z_{10}	CCGACAAACC
O_{10}	TCAGAGCGCC
O_{15}	TGGCGTCCTT

PCR amplification reactions contained 10 mM Tris-HCl with PH 8.8 at 25° C, 50mM KCl, 2.0 mM $MgCl_2$, 200 µM of each of NTP, 0.2 µM 10-mer primer, 10 ng template DNA and 0.5 units of Taq DNA polymerase (promega). Amplifications were performed for 40 cycles of 40 s at 94° C, 1 min at 36° C, 1 min at 72° C, and ending with 6 min at 70° C. Amplified DNA products were separated on 14 g l-1 agarose in 1 X TAE buffer (40 mM Tris base, pH 8.0, 20 mM glacial acetic acid, 2 mM Na EDTA). The run was performed for one hour at 100V in Pharmacia submarine, then stained in an ethidium bromide soak. Bands were detected on UV-transilluminator and photographed by a Polaroid camera.

The fragment sizes of the amplification products were estimated from the gel by comparison with standard molecular markers (M1 and M2). M1 refers to DNA ladder with 10000, 8000, 6000, 5000, 4000, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250 bp while M2 refers to combines digests from Lambda / Hind III and Ø x 174 / *Hae* III with Dna fragments 23130, 9416, 6557, 4361, 2322, 2027, 1353, 1078, 872, 603 and 310 bp.

Statistical analysis:

All data of yield and yield components (number of open bolls / plant, boll weight (g), lint yield / plant (g) and seed cotton yield / plant (g), were subjected to the statistical analysis according to Steel and Torrie (1980). Means were computed according to Duncan's (1955), multiple range test.

DNA amplification profiles of six cotton varieties were compared with each other and bands of DNA fragments were scored as present 1 or absent 0. the data for all five primers was used to estimate genetic distances on the basis of the number of shared amplification products (Nei and Li, 1979). A dendrogram based on dissimilarity coefficients was generated by using the unweighted pair group method of arithmetic means (UPGMA).

RESULTS AND DISCUSSION

The data of mean performance of seed cotton yield and its main attributes for the six cotton varieties under saline conditions of Wadi Sudr, during both growth seasons (2000 and 2001), were represented in (Table 2). As expected, a significant variability was observed among the studied varieties for yield and its attributes. Most long staple varieties attained higher significant values than extra long staple varieties where Giza 90 had the highest significant number of open bolls / plant, lint yield / plant and seed cotton yield / plant, Giza 83 ranked in the second order followed by Dendera, whereas Giza 77 then Giza 45 had the lowest mean values. The same trend was clearly observed in boll weight but Dendera attained the highest values followed by Giza 83 then by Giza 90, Giza 77 had the lowest value. Similar results were obtained by El-Razaz *et al.* (1997) and Afiah and Ghoneim (1999). So, long staple varieties especially Giza 90, Giza 83 and Dendera considered the more adapted varieties and could be occupy a considered positions in breeding program for improving adaptation to saline conditions of Wadi Sudr. In the same respect, Afiah and Ghoneim (1999) reported that Giza 83 is the best cultivars under saline conditions of Ras Sudr South Sinai.

Also, data presented in Table (2) illustrate a significant correlation between seed cotton yield / plant and its main attributes for six cotton varieties examined, and exhibited positive correlation coefficient values (r) with lint yield / plant, number of open bolls / plant and boll weight (0.991, 0.883 and 0.513 respectively). Afiah and Ghoneim, (2000) previously obtained to great extent similar results.

Table (2): Mean performance of seed cotton (*Gossypium barbadense* L.) yield and its main attributes grown under saline conditions

Variety	Number of open bolls/plant	Boll weight/gm	Lint yield/plant	Seed cotton yield/plant
Giza 77	4.849 d#	2.087 b-c	3.595 c	10.959 c
Giza 45	4.753 d	1.790 d	3.341 c	9.463 d
Giza 83	7.157 b	2.239 b	6.578 a	17.830 a
Giza 85	5.324 c-d	2.038 c-d	3.836 c	10.184 c-d
Giza 90	8.240 a	2.081 b-c	7.385 a	18.785 a
Dendera	5.902 c	2.622 a	5.564 b	15.360 b
r.	0.883 **	0.513 *	0.991 **	

*and ** : Denote significance at 0.01 and 0.05 levels of probability, respectively.
#: Values followed by the same letter(s) are not significantly different at 0.05 level of Duncan's multiple range test.
r: Simple correlation coefficient between seed cotton yield / plant and its main attributes.

Out of the oligonucleotide primers screened in this study five primers detected a scorable polymorphism between the six cotton varieties. Such primers amplified a total of 55 DNA fragments, out of these amplified fragments 22 (40 %) were monomorphic. The rest of bands (33) that equal 60 % were polymorphic. The number of amplification products generated by each primer within the cotton varieties varied from 33 to 73 %. The levels of polymorphism were differed among different primers (Figs. 1 and 2). Thus these results seemed to discriminate genetic diversity between the studied cotton varieties. Cantrell and Davis (1993), Multani and Lyon (1995) and Tatineni *et al.* (1996) demonstrated that genetic diversity among different cotton genotypes and varieties can be distinguished by using RAPD markers.These results were confirmed by statistical analysis. A similarity matrix was generated from the RAPD results using Nei and Li's (1979) coefficient that clearly shown in Table 3. These similarity coefficients were used to generate a dendrogram (Fig. 3) by UPGMA analysis in order to determine the similar group of different varieties. From the similarity matrix, the least similar variety was Giza 77. Its similarity ranged from 60 to 54 % with Giza 85 to 54 % with Dendera. The low similarity of Giza 77 with other studied varieties may be due to the fact that it shared a very little genetic background one or two ancestors with other studied cotton varieties, it shared (Table 1). In the dendrogram the extra long staple which has a significant lowest yielding ability and most of its attributes than the other cotton varieties, Giza 77 did not cluster with any other variety tested and can be easily distinguishable (Fig. 3). Giza 85 and Giza 45 were grouped together thate, their similarity coefficient was 80 % and attained lowest value 60 % and 56 % with Giza 77 respectively (Table 3). In addition had significant lower performance of seed cotton as well as and its attributes under saline conditions as compared with Giza 90, Giza 83 and Dendera.

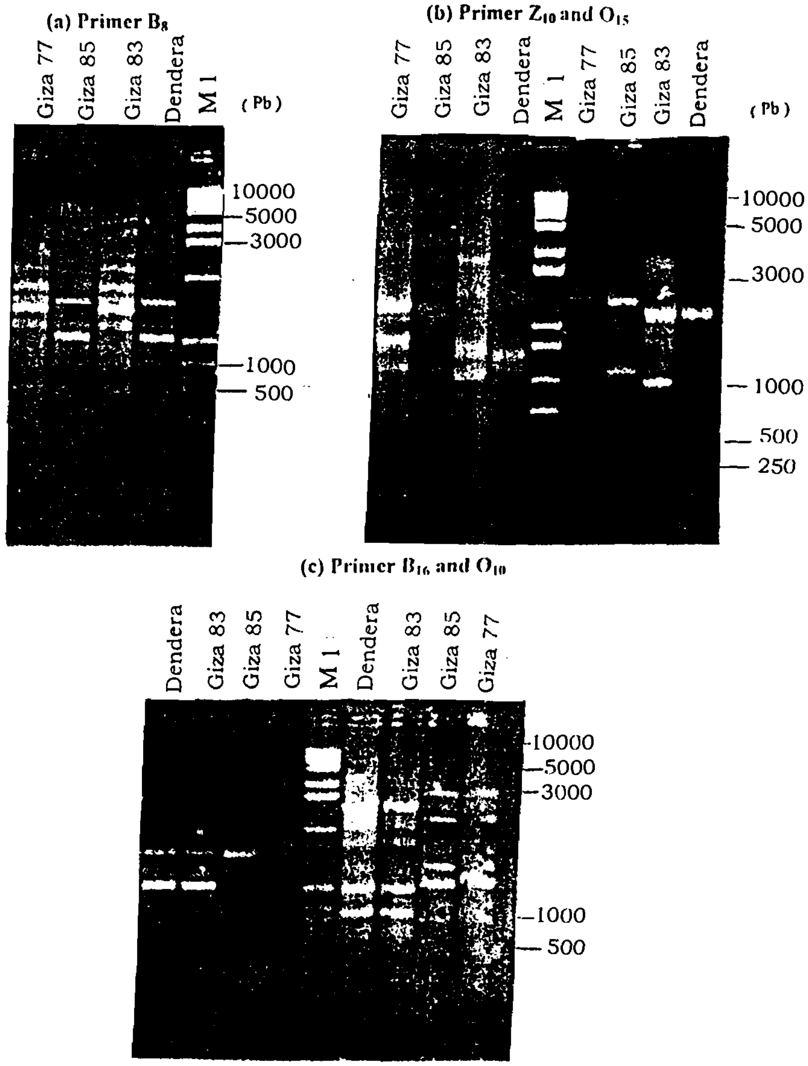
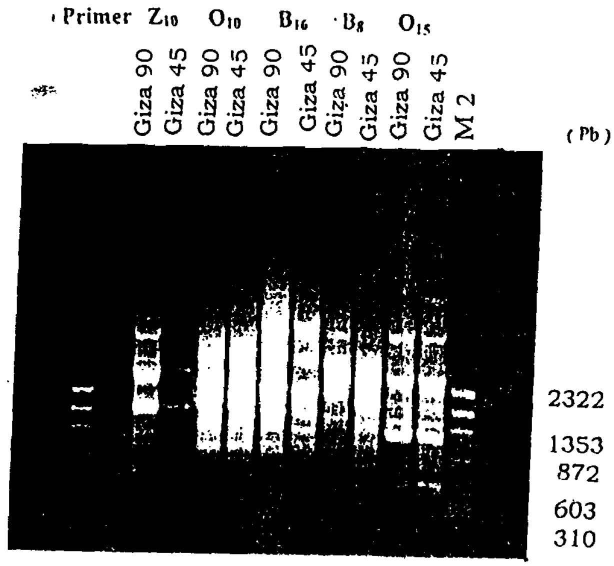


Fig. (1): DNA polymorphism based on RAPD analysis for the six cotton (*Gossypium barbadense* L.) varieties Giza 45, Giza 77, Giza 83, Giza 85, Giza 90 and Dendera, using primers B_8 (a), Z_{10} and O_{15} (b), B_{16} and O_{10} (c). M1 and M2 refer to DNA standards.

Fig. (2): DNA polymorphism based on RAPD analysis for the six cotton (*Gossypium barbadense* L.) varieties Giza 45 and Giza 90, using primers B_8, Z_{10} and O_{15}, B_{16} and O_{10}. M2 refer to DNA standards.



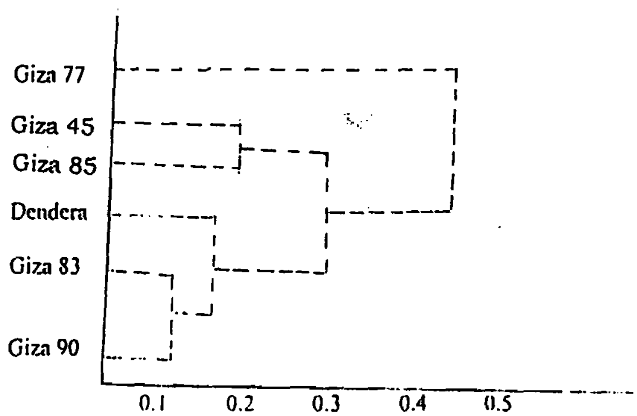


Fig. (3): Dendrogram demonstrating the genetic distance among the six cotton (*Gossypium barbadense* L.) varieties based on RAPD analysis.

Table (3): Similarity coefficient across RAPD analysis of the six cotton (*Gossypium barbadense* L.) varieties.

Case	Giza 45	Giza 77	Giza 83	Giza 85	Giza 90
Giza 77	0.56±0.03				
Giza 83	0.70±0.04	0.57±0.04			
Giza 85	0.80±0.04	0.60±0.02	0.76±0.05		
Giza 90	0.72±0.03	0.55±0.05	0.89±0.04	0.68±0.05	
Dendera	0.70±0.02	0.54±0.03	0.79±0.06	0.61±0.06	0.87±0.04

Similarity matrix data revealed that variety Giza 90 was 89 % and 87 % similar to both Giza 83 and Dendera, respectively. Giza 90, Giza 83 and Dendera grouped together (Fig. 3), also, attained a significant highest performance of yield and its attributes under saline conditions. Giza 90 drived from cross between Giza 83 and Dendera (Table 1).

The genetic similarity of the studied six cotton varieties ranged between 89 % and 54 % on the basis of the RAPD data and their genetic base looks narrow (Iqbal *et al.*, 1997). These results were in accordance with the known genetic make up (pedigree) of cotton varieties (Table 1). New Egyptian cotton varieties produced by hubridization of homozygotic local varieties inorder to create genetic variability. The old local varieties were of Ashmouni type, selected by the keen eyes of the cotton growers (Abdel-Tawab *et al.*, 2001).

In conclusion, the cotton varieties used in this study were elite commercial varieties. The results of their different adaptability (mean performance of yield and its attributes) among the studied cotton varieties, as well as the genetic relationships were in accordance with the earlier studies. Also,The results from this investigates fucing to great extent the usefulnes of RAPD markers for the evaluation of the genetic similarities for the studied cotton varieties. Moreover the results obtained from this analysis could be used for the selection the suitable parents for breeding program for improving adaptability to saline conditions of Wadi Sudr. Accordingly long staple variety Giza 90 is the more adapted cotton variety to saline conditions of Wadi Sudr whereas, extra long staple variety Giza 77 was the least adaptation, and there was adequate genetic variation between cotton varities. Finally, Breeders can be hybridize the two varieties in order to produce new genotypes characterized with high fiber qualities and adapted to saline conditions.

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تحسين الأداء الوراثى لبعض أصناف القطن المصرى تحت الظروف الإجهاد البيئى لوادى سدر جنوب سيناء

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أقيمت تجربتين بمحطة بحوث راس سدر الزراعية خلال الموسمين المتتالين ٢٠٠٠، ٢٠٠١ لدراسة أداء (تكيف) ستة أصناف من القطن المصرى تحت الظروف الملحية لوادى سدر. و كذلك لتقييم التنوع الوراثى و العلاقات الوراثية بين هذه الأصناف باستخدام تحليل الأشكال المتعددة للحمض النووى ال DNA (RAPD). و قد حصلت الأصناف طويلة التيلة جيزة ٩٠ ثم جيزة ٨٣ و تبعهم دندرة على قيم معنوية عالية للمحصول و مكوناته لأقلمتهم العالية للملوحة. بينما قل الإنتاج بصورة معنوية للصنفين فائقى طول التيلة جيزة ٧٧ ثم جيزة ٤٥ و بذلك فهم اقل تأقلما للظروف الملحية.

تم استخدام خمسة بادئات عشوائية لتكبير الحمض النووى الديوكسى ريبوزى ال DNA بواسطة تفاعل البلمرة التسلسلى و إنتاج RAPDs.وقد اكتشف الخمس بوادئ تعدد أشكال ال DNA المكبر فى كل الستة أصناف المستخدمة و وجد ان النسبة المئوية من أشرطة ال DNA المكبرة عديدة الأشكال تم إنتاج مصفوفة التشابه من نتاج ال RAPD باستخدام معامل Nei and Li (1979) .و كان الصنف جيزة ٧٧ اقل تشابها مع بقية الأصناف بينما تشابه الصنفان جيزة ٩٠ و جيزة ٨٣ تشابها كبيرا. كما أوضح الشكل الشجرى المنتج بواسطة تحليل المجموعات الثنائية للمتوسط الحسابى (UPGMA) ان الأصناف الأكثر تحملا لظروف الملوحة الطويل التيلة جيزة ٩٠، جيزة ٨٣ و دندرة قد تم اصطفافهم مع بعضهم كما اصطف الصنفان الأقل معنويا فى الإنتاج جيزة ٤٥ و هو فائق فى طول التيلة و جيزة ٨٥ طويل التيلة مع بعضهم بينما اصطف الصنف فائق فى طول التيلة و الأقل أقلمة و إنتاجا جيزة ٧٧ منفردا.

ان النتائج المتحصل عليها يمكن استخدامها فى اختيار الأباء بأسلوب علمى لتنفيذ برامج تربية لتحسين نوعية الألياف و الانتاج و تحسين الأقلمة للظروف الملحية فى وقت قصير بدون الحاجة الى زراعة الاباء و اختبارها فى الأجيال المتتالية.