J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 4 (5): 217 - 227, 2013

IMPACT OF PLANT BREEDING ON GENETIC DIVERSITY OF THE EGYPTIAN COTTON (*Gossypium barbadense* L.) VARIETIES AS REVEALED BY RAPD MARKERS

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ABSTRACT

Genetic diversity changes in cotton have been studied using different molecular markers, but little is known about the impact of plant breeding on the cotton genome. The objective of the present study was to assess diversity changes in Egyptian cotton varieties released from 1920 to 1998 using 19 RAPD markers. The total number of fragments ranged from 65 to 90 with an average 82.8. Fragment frequency ranged from 0.157 to 0.217 with an average of 0.20. The number of private fragments ranged from one to six. These results suggest that the Egyptian cotton breeding has reduced genetic diversity in the cotton genome. The Shanon information index ranged from 0.125 to 0.209 with an average of 0.147. The expected heterozygosity values ranged from 0.086 to 0.143. The Nei genetic distance among the five released periods based on RAPD markers ranged from 0.099 to 0.269 with an average 0.164. The five cotton breeding periods were classified into four major groups. The PCOA1 and PCOA2 axis explained a reasonable amount of variation 41.68% and 74.04%, respectively. A significant correlation coefficient between gene diversity and the number of fragments was high, r = 0.985 ($P > 0.01$). Furthermore, analysis of AMOVA revealed non-significant genetic variance among breeding periods. The proportion of RAPD variation accounted by decadal grouping was low (Φ_{PT} = 0.066, p = 0.230). A genetic shift was observed in the cotton varieties released over the five breeding periods. These results illustrate the impact of the cotton breeding on the Egyptian cotton genome.

Keywords: Cotton (Gossypium barbadense L.); Genetic shift; genetic relationship; RAPD marker; Breeding periods.

INTRODUCTION

Genetic diversity changes in cotton (*Gossypium barbadense* L.) germplasm have been studied using several different kinds of molecular markers, but the impact of modern plant breeding on cotton genetic diversity in Egyptian regional breeding programs has been poorly understood. Some studies have suggested the diversity reduction accompanying plant improvement has been limited (*Donini et al. 2000; Christiansen et al. 2002; Khan et al. 2005; Reif et al. 2005*).

For example, (*Khan et al. 2009*) assessed the genetic diversity in 40 cotton cultivars grown in the Pakistan from 1914 to 2005 and observed just a qualitative shift in genetic diversity over time. Other studies have demonstrated the reduction of allelic counts in some improved gene pools of cotton (*Iqbal et al., 2001; Zhang et al. 2005*).

Breeding methods and selective pressures may differ in various breeding programs and significant diversity reduction should not be expected to occur in every improved gene pool of cotton. Also, all the studies used different markers of unequal quality and diversity measurements of variable accuracy (*Mohammadi and Prasanna 2003*), thus making the generalization of the findings difficult. Moreover, bias may exist in the diversity comparison of unequally sized groups and in the selection of less representative cultivars for different breeding periods (*Fu et al. 2003*). Thus, it is important to recognize these limitations for an informative study of genetic diversity changes.

Characterization of plant germplasm using molecular techniques has an important role in the management and utilization of plant genetic resources (*Karp, 2002*). It can also enhance plant breeding in selection of diverse parents to widen the breeding gene pool (*Fu, 2006*). Efforts have been made to characterize cotton germplasm using allozymes (*Wendel et al., 1992*), restriction fragment length polymorphism (RFLP) (*Wendel and Brubaker, 1993*), random amplified polymorphic DNA (RAPD) (*Multani and Lyon, 1995; Iqbal et al., 1997, Lu and Myers, 2002*), amplified fragment length polymorphism (AFLP) (*Iqbal et al., 2001; Rana et al., 2005*) and simple sequence repeat (SSR) markers (*Liu et al., 2000; Reddy et al., 2001; Zhang et al., 2005; Lacape et al., 2007*). These characterizations have provided useful information for understanding the genetic diversity and structure of various cotton gene pools found in different geographic regions. This information has been incorporated into effective management of cotton germplasm in some cotton breeding programs for control of genetic diversity. In general, low levels of genetic diversity have been found in modern cotton cultivars, which is consistent with the hypothesized narrow genetic base of upland cotton germplasm used in breeding (*Meredith, 2000*).

In 2009, (*Khan et al. 2009*) initiated an assessment on the genetic diversity of 40 commercial Pakistan cotton cultivars released since 1914-2005 using 34 simple sequence repeat (SSR) markers. This study revealed a significant slightly more variation for cultivars released after 2000 than those released earlier, suggesting that genetic diversity has been maintained in Egyptian's long term cotton breeding program. Little is known if such a selective impact has also affected the transcribed segments of the cotton genome, as SSR markers presumably are neutral (or frequently non-coding) and may represent different regions of the cotton genome. (*Khan et al 2009*) inspired a repeat of the initial assessment with the hope of determining the generality of the selective impact of plant breeding, particularly on the transcribed segments of the cotton genome. A loss of function-associated alleles is of most concern, as narrowing the base of functional genes may reduce the allelic diversity contributing to an adaptive or economic value. The overall objective of this study was to analyze the patterns of genetic variability in 11 Egyptian cotton varieties released from 1920 to 1998 using 19 RAPD markers. Specifically, the RAPD variability was analyzed with respect to breeding period with the aim to determine the impact of plant breeding on the transcriptional segments of the Egyptian cotton genome.

MATERIALS AND METHODS

Plant materials

According to cotton-growing regions, geographic origin and released time from 1920s to 1998s, 11 Egyptian-bred cotton varieties were used in the study (Table 1). Since cotton breeding was conducted mainly in the second half of the 20th century, most of the varieties tested were registered within the past 78 years. Because of a rapid increase in breeding efforts in 1970s, 1980s and 1990s, a large amount of varieties were taken from these time periods. The eleven Egyptian cotton varieties were classified into five breeding periods as follow (1920, 1943-1950, 1971-1975, 1980-1982 and 1993-1998). All of the varieties were provided by the Cotton Institute, Agriculture Research Center (ARC), Ministry of Agriculture, Egypt.

Table (1): Eleven Egyptian cotton varieties with parentage and year of released.

No	Cultivar name	Parentage	Released Time
1	Ashmony	MahoJumel x *G.barbadense* from south America	1920
2	Dandara	Giza 31= Selected from Giza 3	1943
3	Giza 45	Giza 28 x Giza 7	1950
4	Giza 70	Giza 59A x Giza 51B	1971
5	Giza 75	Giza 67 x Giza 69	1975
6	Giza 76	Menoufi x Pima	1980
7	Giza 77	Giza 70 x Giza 68	1982
8	Giza 80	Giza 66 x Giza 73	1981
9	Giza 85	Giza 67 x CB 58	1993
10	Giza 88	(Giza 77 x Giza 45) B	1995
11	Giza 89	Giza 75 x S. 6022	1998

DNA isolation

Germinated cotton seeds from each variety were cultured in the growth chamber at 25-28°C for about one week. Total genomic DNA was extracted using about 3 g of fresh cotyledon tissue from 15-20 plants of each genotype according to (*Paterson et al. 1993*) Protocol.

PCR and RAPD primers

Random primers (University of British Colombia, UBC) were dissolved in sterilized distilled water at a concentration of 15 ng/µl. Ninteen primers belonging to UBC were used for PCR amplifications. Amplifications were carried out in a 25µl reaction volume containing 10mM Tris-HC1 (pH 8.3 at 25°C), 50mM KCl, 3.0mM MgCl 2 , 0.1 mM each of dATP, dGTP, dCTP and dTTP, 1 unit of *Taq* DNA polymerase (Perkin Elmer, Norwalk, Conn.), 0.001% gelatin (Sigma, St-Louis, Mo.), 50 ng of template DNA and 30 ng of primer. The amplifications were carried out in a Perkin Elmer Thermal Cycler 480, programmed for a first denaturation step of 3 min at 94°C followed by 45 cycles of 94°C for 1 min, 40°Cfor 1 min and 72°C for 2 min. After the

completion of 45 cycles, the reactions were kept at 72°C for 5 min and then held at 4°C until the tubes were removed. PCR products were separated on a 1.2% agarose gel with ethidium bromide in the gel using 1x Tris Borate EDTA (TBE) buffer.

Band scoring and data analysis

Amplification profiles of eleven cotton varieties were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). The data for all the nineteen primers was used to estimate the similarity on the basis of the number of shared amplification products (*Nei and Li 1979*). A dendrogram based on similarity coefficients was generated by using the unweighted pair group method of arithmetic means (UPGMA). Genetic similarity (GS) was estimated based on (*Nei and Li's coefficient 1979*) using the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) Version 2.11a software package (*Rohlf 1993*). Genetic diversity, number of fragments and number of private fragments, Shanon information index, expected heterozygosity, and Nei 1972 distance matrix (*Nei 1972*) were calculated using Power Marker (*Liu and Muse 2005*). AMOVA was estimated using Arlequin (*Excoffier et al. 2005; Labate 2000*). The software PAST (PAlaeontological STatistics) (*Hammer et al. 2001*) was used to perform both the Principal Coordinate Analysis (PCoA) superimposed by the minimum spanning tree and the cluster analysis using Unweighted Pair-Group Method using Arithmetic averages (UPGMA) based on Nei genetic distance matrix.

RESULTS AND DISCUSSION

RAPD Polymorphism

The total number of fragments ranged were 90 for 1943-1950 = 1980-1982 > 1993-1998 (86) > 1971-1975 (83) > 1920 (65) with an average of 82.8 (Table 2). Fragment frequency ranged from 0.157 for the breeding period 1920 to 0.217 for the two breeding period 1943-1950 and 1980-1982 with an average of 0.200. The number of private fragments ranged from zero for breeding period 1920 to six for the breeding period 1980-1982 with an average of 2.2, indicating that the genetic diversity was shifted due to the breeding program. These results suggest the Egyptian cotton breeding has reduced genetic diversity in the cotton genome. Significant reduction in number of private fragments for 1920 breeding period over the five breeding periods were identify because these breeding period include only one variety.

The Shanon information index ranged from 0.125 for the breeding period 1971-1975 to 0.209 for the breeding period 1943-1950 with an average of 0.147. The expected heterozygosity values ranged from 0.086 for the breeding period 1971-1975 to 0.143 for the breeding period 1943-1950 with an average 0.100.

Table (2): Summary statstics of RAPD fragments and heterozygosity estimates across five Egyptian cotton breeding released periods

Release period	1920	1943-1950	1971-1975	1980-1982	1993-1998	Average
Total Number of fragments	65	90	83	90	86	82.8
Fragment frequency (Frequency >= 5%)	0.157	0.217	0.200	0.217	0.208	0.200
Number of private fragments	0	3	1	6	1	2.2
[1]Information index (SE)	0.000 (0.000)	0.209 (0.027)	0.125 (0.023)	0.207 (0.027)	0.194 (0.026)	0.147 (0.021)
[2]Mean H_e (SE)	0.000 (0.000)	0.143 (0.018)	0.086 (0.016)	0.142 (0.019)	0.132 (0.018)	0.100 (0.02)

[1]Shanon information index
[2]Expected Heterozygosity.

Genetic distance for RAPD markers among breeding periods

Nei pair wise similarity estimates among breeding periods were calculated and have been presented in (Table 3). Genetic distance among the five breeding periods ranged from 0.099 (1971-1975 and 1993-1998) to 0.269 (1920 and 1980-1982) with an average of 0.164. Other genetic diversity estimates in impact of plant breeding on genetic diversity have been reported using different molecular markers (*Fu et al. 2006, Khan et al. 2009 and Zhang et al. (2011*).

Table (3): Nei Genetic distance among five Egyptian cotton breeding released periods based on RAPD data

	1920	1943-1950	1971-1975	1980-1982	1993-1998
1920	0.000				
1943-1950	0.148	0.000			
1971-1975	0.213	0.103	0.000		
1980-1982	0.269	0.176	0.213	0.000	
1993-1998	0.187	0.080	0.099	0.154	0.000

Genetic diversity among Egyptian cotton breeding periods using RAPD markers

A dendrogram derived from UPGMA cluster analysis based on the genetic similarity coefficient matrix for the five breeding periods was constructed basically; all cotton breeding periods could be distinguished. The genetic distance for all breeding periods ranged from 9.9% to 26.9% with an average 16.4%. On the basis of Nei coefficient, the five cotton breeding periods can be classified into four major groups (Figure 1), (i) group one includes the two cotton breeding period 1920 and 1980-1982; (ii) group two includes only the cotton breeding period 1971-1975; (iii) group three include the cotton breeding period 1993-1998 and (iv) group four includes the cotton breeding period 1943-1950. In general the cotton breeding period 1943-1950 was the most genetically diversified from the other cotton breeding periods and could be important sources for new cultivar development if they differ in useful agronomic traits.

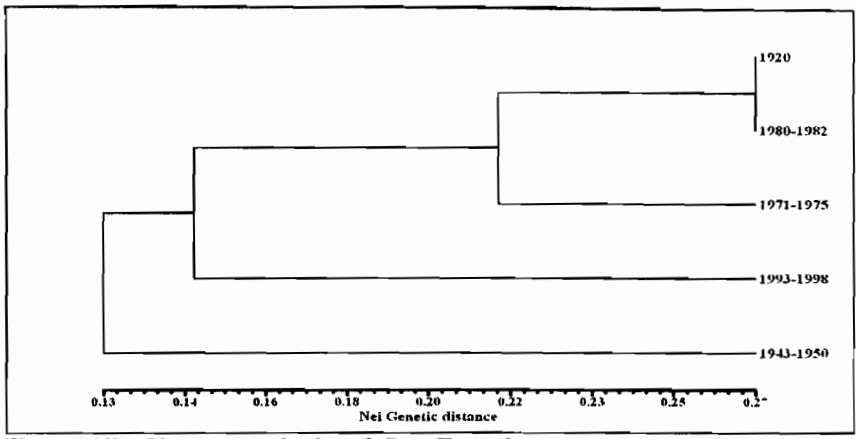

Figure (1): Cluster analysis of five Egyptian cotton breeding released periods represent 11 cotton varieties based on RAPD data using Nei genetic distance.

Shift of genetic background over time

To visualize the genetic associations of various decadal groups of Egyptian cotton varieties, groupwise varieties similarities with an average 82.8 fragments were calculated and analyzed. The analysis revealed a shift of genetic background in the Egyptian cotton varieties released from 1920 to 1998. The associations of the cotton varieties groups representing the five breeding periods are shown in Figure (1), and two major clusters were found and appeared to be associated with the two major types of breeding effort over time. Breeding efforts focused on high yield such as i.e., seed cotton yield, number of bolls per plant, lint cotton yield per plant, pest resistant, early maturing and quality improvement. Such association, however, still needs to be empirically determined. Genetically, these efforts increased the similarity (or genetic relatedness) of Egyptian cotton varieties within breeding periods, resulting in a gradual diversity shift. This shift was more obvious in Figure (2) where associations of individual cotton varieties were assessed based on the principle coordinate (PCO) analysis. The first two PCO axes explained a reasonable amount of variation (41.68 and 74.04%, respectively).

When the Egyptian cotton varieties were labeled according to breeding periods, the cultivars released later were gradually shifting away from early introductions from the right to the left of Figure (2). Such genetic shift reflected well the change in breeding focus over time as identified by old varieties.

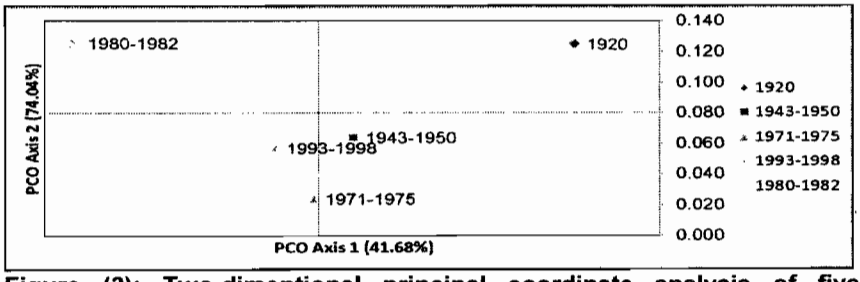

Figure (2): Two-dimentional principal coordinate analysis of five Egyptian cotton breeding release periods based on RAPD markers.

On the bases of Nei coefficient for RAPD markers, the five cotton breeding periods were classified into four major groups. However, based on Euclidean distance for agronomic traits, the five Egyptian cotton breeding periods were classified into three major groups. The PCOA1 and PCOA2 axis explained a reasonable amount of variation 41.68% and 74.04%, respectively. A significant correlation coefficient between gene diversity and the number of fragments was high, r = 0.985 (*P> 0.01*). Furthermore, analysis of molecular variance (AMOVA) revealed non-significant genetic variance among breeding periods (Table 4). The proportion of RAPD variation accounted for by decadal grouping was low (Φ_{PT} = 0.066, p = 0.230). A genetic shift was observed in the cotton varieties released over the five breeding periods reflecting different breeding efforts. These results illustrate the impact of the cotton breeding on the Egyptian cotton genome. These findings, along with those from genomic RAPD markers, suggest the Egyptian cotton breeding programs have reduced genetic diversity in the Egyptian cotton varieties.

Table (4): Analyses of molecular variance (AMOVA) of four released periods (1920-1950, 1971-1975, 1980-1982, and 1993-1998) using Φ-statistics based on RAPD data

Source	df	SS	MS	Estimated variance	%
Among groups	3	48.424	16.141	0.960	7
Within groups	7	94.667	13.524	13.524	93
Total	10	143.091		14.484	100

Φ_{PT} = 0.066 (p= 0.230)

Implications for Egyptian cotton germplasm conservation and breeding

The findings of this study have several practical implications. First, the overall genetic diversity residing in these cotton varieties was low, implying the need for continued effort to widen the diversity range both for Egyptian cotton germplasm conservation and future breeding. Second, the varieties released after 1980 displayed slightly more RAPD variation than those released earlier, suggesting that genetic diversity has been maintained in Egyptian's long-term cotton breeding programs. Third, this study revealed

two major clusters of cotton breeding periods and identified the genetically most distinctive cotton varieties. The findings on genetic relationship and distinctiveness are useful for parental selection of diverse plants for Egyptian cotton breeding program. Fourth, the characterization of cotton varieties using RAPD markers generated not only essential information for understanding genetic diversity of elite Egyptian cotton germplasm, but also provided a useful guide for selecting specific germplasm with distinct genetic background for diversifying Egyptian cotton breeding program.

In summary, This RAPD analysis revealed that the genetic diversity of the Egyptian cotton varieties released since 1920 was relatively low, but has been maintained over the long-term breeding. The analysis also generated information on genetic relationships and identified genetically unique Egyptian cotton varieties. These findings are useful for conserving Egyptian elite cotton germplasm and developing future cotton breeding programs in Egypt. Also, this study represents the attempt using molecular markers derived from transcribed sequences to assess the genetic diversity changes in an improved cotton gene pool. A significant reduction in allelic count was observed at different breeding period for the cultivars released after 1982. Genetic drift, however, might also have contributed to the allelic changes, as the lost alleles were mostly rare. Question remains whether all the lost RAPD bands are associated with undesirable traits. The findings presented here, along with those previously reported from genomic SSRs markers (*Fu et al. 2005*), support the hypothesis that modern plant breeding is reducing genetic diversity (*Fu et al. 2003*) in wheat breeding programs. Conservation of genetically diverse germplasm is justified and useful for long-term breeding efforts (*Duvick 1984; Swanson 1996; Tripp 1996*). Continuous diversification of plant breeding materials is warranted to ensure that the plant improvement continues to be sustainable in the future (*Reif et al. 2005*). Developing effective indicators for genetic diversity of cultivated plants not only enhances the monitoring of genetic changes in improved gene pools, but also the effort of germplasm conservation and utilization (*Fu et al. 2005*). These findings, along with those from genomic RAPD markers, suggest the Egyptian cotton breeding programs have reduced genetic diversity in the Egyptian cotton.

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تأثير تربية النبات على التنوع الوراثى لأصناف القطن المصرى بايضاحها بالمعلم الجزيئى RAPD

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تم دراسة تغييرات التنوع الوراثى فى القطن المصرى باستخدام المعلمات الجزيئية المختلفة ولكن يعرف القليل عن تأثير تربية النبات على جينوم القطن. وعليه فالهدف من هذه الدراسة هو تحديد تغييرات التنوع الوراثى فى أصناف القطن المصرى فى الفترة من ١٩٢٠ الى ١٩٩٨ باستخدام ١٩ معلم جزيئى RAPD . تم الحصول على ٦٥ الى ٩٠ شظية كعدد كلى بمتوسط ٨٢.٨ شظية. اتضح أن تكرار الشظايا يتراوح من ٠.١٥٧ الى ٠.٢١٧ بمتوسط ٠.٢ بينما تتراوح عدد الشظايا الخاصة من ١ الى ٦. وأشارت النتائج الى أن برامج تربية القطن المصرى أدت الى خفض التنوع الوراثى فى جينوم القطن. تراوح معامل شانون من ٠.١٢٥ الى ٠.٢٠٩ بمتوسط٠.١٤٧ كذلك تراوحت قيم عدم التماثل الوراثى من ٠.٠٨٦ الى ٠.١٤٣ كما أن المسافة الوراثية Nei تراوحت من ٠.٠٩٩ الى ٠.٢٦٩ بمتوسط ٠.١٦٤ .

أمكن تقسيم الخمس فترات زمنية المدروسة الى أربع مجاميع رئيسية. أوضح PCOA1 و PCOA2 كميه معقولة من التباين ٤١.٦٨% و ٧٤.٠٤% على التوالى. اتضح وجود ارتباط معنويا عاليا بين التنوع الوراثى وعدد الشظايا المتحصل عليها حيث أن معامل الأرتباط كان ٠.٩٨٥ . كذلك أوضح تحليل AMOVA عدم وجود أختلافات وراثية بين فترات التربية.

قام بتحكيم البحث

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