Molecular Characterization of Some Egyptian cotton Varieties

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ABSTRACT

Cotton plant belongs to the genus *Gossypium* which is considered as the first important economic crop in Egypt. The present study aims to characterize five Egyptian cotton varieties (Giza(G) 92;Giza(G) 93; Giza(G)86; Giza(G)87and Giza(G)88) at biochemical and molecular level. Using ISSR; SSR and RAPD-PCR analyses, a total of 159 polymorphic fragments were recorded with similarity of 40; 23.5 and 38%, respectively, for the five varieties. The Phylogenetic relationships of the five Egyptian cotton varieties based on DNA molecular data (RAPD + ISSR + SSR) indicated two groups with 56% similarity; the first group had (G88); However, G93 and G86 with homology percentage of 81%, and both of them in relation to G87 variety with approximately 65.5% similarity.

Keywords: Cotton, Molecular Markers, ISSR, SSR, RAPD-PCR

INTRODUCTION

Cotton is an important natural plant of the genus *Gossypium* and a shrub native to tropical and subtropical regions. It is fluffy staple fiber that grows in a boll or protective capsule around seeds and used to make a soft breakable textile. The fiber is almost pure cellulose and aid in seed dispersal, according to Basra and Malik (1984) as well as Ruan and Chourey (1997).

It is independently domesticated around the old and new worlds including Americas; Africa and India. Four commercially-grown species of cotton had been domesticated in antiquity. *Gossypium hirsutum* – upland cotton, native to Central America; Mexico; Caribbean and Southern Florida (90% of world production). *Gossypium barbadense* – known as extra-long staple cotton, native to tropical South America (8% of world production). *Gossypium arboreum* – tree cotton, native to India and Pakistan (less than 2% of world production). *Gossypium herbaceum* – Levant cotton, native to Southern Africa and Arabian Peninsula (less than 2% of world production). (Edwards and Mirza 1979)

The *Gossypium* genus comprises about 45 diploid species with 26 chromosomes and 5 allotetraploid species (tetraploids derived following hybridization of two diploids) with 52 chromosomes (Brubaker *et al*.,1999a; Esmail et al.,2017). Based on chromosomal similarities, *Gossypium* species commonly are grouped into eight diploid genomic groups (A - G and K) and one tetraploid genomic group (Edwards and Mirza, 1979 and Endrizzi *et al.*, 1985 and Stewart, 1995). Two economically important cultivated tetraploid species of *G. hirsutum* (Upland cotton) and *G. barbadense* (Caribbean "Sea-Island"-"Extra Long Staple"; modern "Pima" and "Egyptian"cultivars) dominate world cotton production (Chen *et al.*, 2007).

In the past decades, molecular markers such as biochemical constituents and macromolecules (proteins and DNA) have very rapidly complemented the classical strategies (Weising *et al.,* 1995). Recently, the term DNA fingerprinting/profiling is used to describe the combined use of several single locus detection systems and are being used as versatile tools for investigating various aspects of plant genomes. These include characterization of genetic variability; genome fingerprinting; genome mapping; gene localization; analysis of genome evolution; population genetics; taxonomy; plant breeding and diagnostics (Joshi *et al.,* 1999).

The present study aims to investigate and characterize five Egyptian cotton varieties. To achieve this purpose some physical properties (length; strength; fineness; uniformity….etc.) were measured using High Volume Instruments (HVI). In addition, the phylogenetic relationships of these varieties were designed according to some DNA molecular markers (ISSR; SSR and RAPD) as well as Peroxidase isozymes data.

MATERIALS AND METHODS

MATERIALS

Five different Egyptian cotton (*Gossypium barbadense* L.) genotypes were used in this study: G92,G93,G86, G87 and G88 and were obtained from Sakha station.

Fine chemicals for DNA isolation; polymerase chain reaction (PCR) primers kits and Peroxidase chemicals were used. The following three tables present the primers used for RAPD; ISSR and SSR analyses.

METHODS

High volume instrument (HVI) testing:

HVI 1000 (Uster, Switzerland) was used as standard test methods for measurement of fiber quality traits and the most important physical properties of cotton fiber, i.e. fiber length (mm); fiber strength (g tex–1) and fiber fineness (micronaire).

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Table 1.P rimers used for RAPD-PCR reactions

Primer number	Primer code	Sequence (5`-3`)
1	OPA-02	CAT CCC CCT G
2	OPA-03	TTC GAG CCA G
3	OPA-04	GTG AGG CGT C
4	OPC-03	GGG GGT CTT T
5	OPC-12	TGT CAT CCC C

Table 2. Primers used for ISSR- PCR reactions

Primer number	Primer code	Sequence (5`-3`)
1	OPA-01	CAG GCC CTT C
2	OPA-02	AGG GGT CTT G
3	OPA-03	CTG GGG ACT T
4	OPA-04	TTC CCC CGC T
5	OPA-05	GAT GAC CGC C

Table 3. Primers used for SSR-PCR reactions

Primer	Sequence of forward primers	Sequence of reverse primers
166wmc	CCACCATGGTGCTAATAGTGTC	AGCTCGTAACGTAATGCAACTG
XTXP-8	ACATCTACTACCCTCTCACC	ACACATCGAGACCAGTTG
XTXP-10	ATACTATCAAGAGGGGAGC	AGTACTAGCCACACGTCAC
XTXP12	ATATGGAAGGAAGAAGCCGG	AACACAACATGCACGCATG
XTXP-19	ATACTATCAAGAGGGGAGC	AGTACTAGCCACACGTCAC
XTXP-20	ATACTATGAACAGGGCAGC	AGTGCTAGCCACACGTGAC

Test method for Micronaire reading:

A predetermined mass of loose cotton was placed in the specimen holder and compressed to a fixed volume. The resistance to air flow, using constant pressure compressed air, was measured and the pressure drop across the plug of cotton was expressed as micronaire. The pressure drops associated with micronaire were determined by performing tests on a wide range of cottons, which were previously established as micronaire values.

Test method for fiber length:

Fibers were placed on a comb in such a way that they are caught at random along their lengths to form a beard. The beard was scanned from base to tip. In the Spinlab system, the amount of light passing through the beard was used as a measure of the number of fibers that extend various distances from the comb. In the Motion Control system, the pressure drop across an orifice was used to measure the number of fibers that extend various distances from the comb.

Test method strength:

For the Spinlab system, the measurement of cotton fiber strength and elongation was made by the same apparatus that measures fiber length and length uniformity. For the Motion Control system, the measurement of cotton fiber strength and elongation was made by the Strength Analyzer.

Leaf tissue preparation:

About 0.05-0.1 g young leaves (2.0-2.5 cm in diameter) were harvested; folded and placed in a 1.5 mL microcentrifuge tube. Samples were kept on ice. Liquefied N2 was used to freeze tissues and then grinded to a fine powder in a 1.5 mL microcentrifuge tube with a pellet pestle.

DNA extraction:

CTAB methods. The quality and quantity of extracted DNA were controlled on a 0.6% agarose gel. Electrophoresis was performed in 1 × TBE running buffer, pH 8.0, at 100V.The agarose gel was checked with a Spectrophotometer DV530 (Beckman, Germany). DNA was quantified by fluorometry and adjusted to 20 ng/µL in distilled water.

Random Amplified Polymorphism DNA (RAPD) - Inter-Simple Sequence Repeat (ISSR) - Simple Sequence Repeats (SSR):

The genomic DNA was amplified using the primers in table (1,2 and 3). The amplifications reactions were carried out in final volume of 20µl. Each reaction mixture contained 2µl DNA template (concentration 30ng/µl DNA); 2µl of 10× PCR buffer; 4.5µl of 0.2 mMdNTPs; 2µl of 50mM $MgCl_2$ and 1.5µl of 30 ng/µl of each forward and reverse primer. The amplification was done for initial denaturation step of 5min at 94°C and 35 cycles. Each cycle was applied as denaturation

for 30 s at 94°C; annealing for 30s at 55°C and extension for 1 min at 72°C. Final extension was done for 10 min at 72°C. The amplified samples (each about 10µl) were separated electrophoretically using a denaturing 3% agarose gel, and also polyacrylamide gel. The ethidium bromide (0.5 mg/mL) was used to stain the gel for about 30 min. and 100bp DNA ladder (5µl) was used as DNA marker. The gel was photographed under ultraviolet light, and fragment length was determined graphically by comparison with the DNA ladder marker. Data was scored as 0 for dearth of band and 1 for presence of band

RESULTS

Using the High Volume Instrument "HVI100 M700 USTER". The five Egyptian varieties were measured, and their results were as shown in Table (4)

Different DNA molecular analyses (RAPD; ISSR and SSR) were applied to estimate the genetic diversity among the five Egyptian cotton varieties.

Random Amplified Polymorphic DNA (RAPD) analysis:

Figure (1): DNA fingerprinting of the five Egyptian cotton varieties using different random primers (OPA-02; OPA-03; OPA-04; OPC-03 and OPC-12). M: DNA marker; 1: G92; 2: G93; 3: G86; 4: G87 and 5: G88.

By using the primers in table (1) the numbers of amplified and polymorphic fragments as well as percentages of polymorphism per primer for the five Egyptian cotton varieties are shown in table (5).

Inter-Simple Sequence Repeats (ISSR):

Figure (2): Banding patterns of the five Egyptian cotton varieties using different ISSR primers (OPA-01; OPA-02; OPA-03; OPA-04 and OPA-05). M: DNA marker; 1: G92; 2: G93; 3: G86; 4: G87 and 5: G88.

By using the primers in Table (2) the numbers of amplified and polymorphic fragments as well as percentages of polymorphism per primer for the five Egyptian cotton varieties were as shown in Table (6)

By using the primers in Table (3) the numbers of amplified and polymorphic fragments as well as percentages of polymorphism per primer for the five Egyptian cotton varieties.

The Phylogenetic relationships of the five Egyptian cotton varieties based on DNA molecular data (RAPD + ISSR + SSR) are as shown in Figure (4).

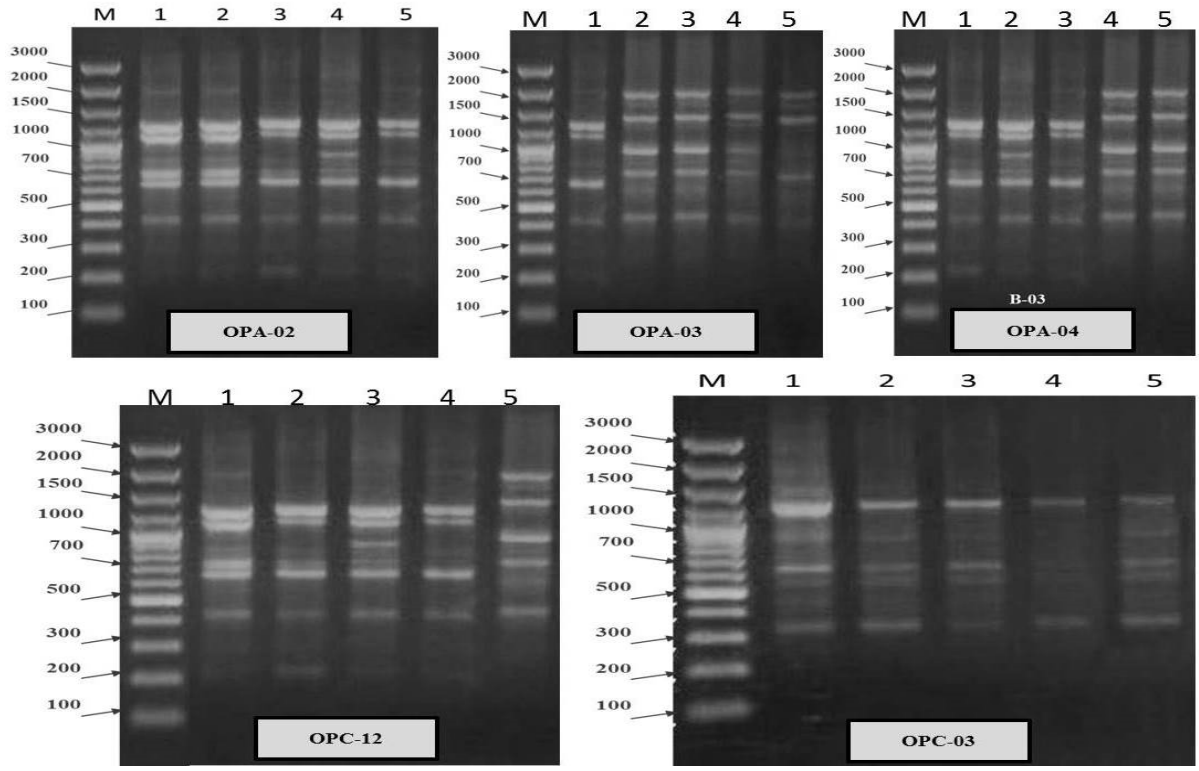

Figure 1. DNA fingerprinting of the five Egyptian cotton varieties using different random primers (OPA-02; OPA-03; OPA-04; OPC-03 and OPC-12). M: DNA marker; 1: G92; 2: G93; 3: G86; 4: G87 and 5: G88.

Table 4. Measurements of some morphological and physical properties for the five Egyptian cotton varieties using HVI1000 M700 USTER

Cotton variety	Length (mm)	Fineness Micronaire reading	Strength (gm/tex)
G92	32-34 Long staple	3.2- 3.99 Fine	42.0- 49.7 Very strong
G93	34.0 - 36.6 Extra-long staple	2.50- 3.04 Extra fine	39.0- 44.9 Very strong
G86	31.0- 33.9 Long staple	3.8-4.99 Fine	39.0-48.9 Very strong
G87	34.47- 35.99 Extra-long staple	3.00 -3.32 Extra fine	40.2- 45.8 Very strong
G88	30.00-35.89 Extra-long staple	3.00- 4.99 Extra fine	35- 44.9 Very strong

Table 5. Number of amplified and polymorphic bands and the percentage of polymorphism based on RAPD

Primer code	Number of amplified bands	DNA length (bp)	Number of polymorphic bands	Percentage of polymorphism (%)
OPA-02	40	210-2500	14	35
OPA-03	45	200-2000	20	44.4
OPA-04	40	210-2000	15	37.5
OPC-03	25	210-2700	8	32
OPC-12	40	320-1500	19	47.5
Total	190		76	40

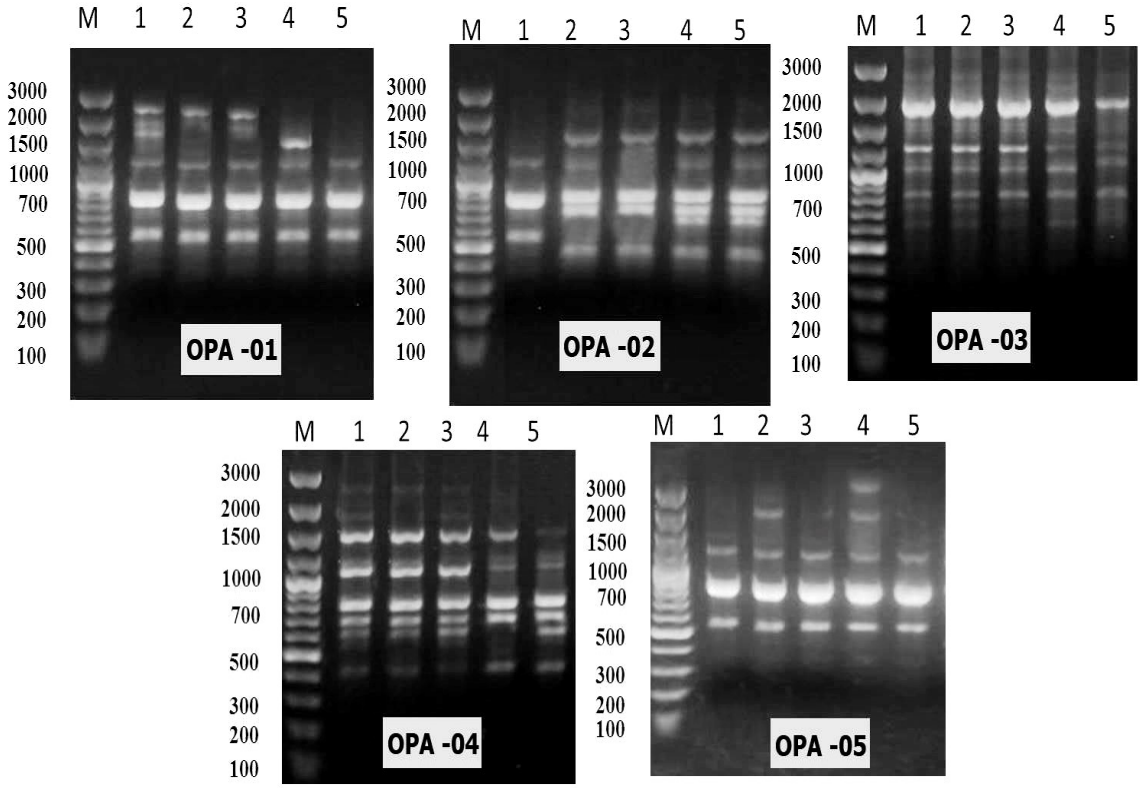

Figure 2: Banding patterns of the five Egyptian cotton varieties using different ISSR primers (OPA-01; OPA-02; OPA-03; OPA-04 and OPA-05). M: DNA marker; 1: G92; 2: G93; 3: G86; 4: G87 and 5: G88.

Table 6. Number of amplified and polymorphic fragments and the percentage of polymorphism per primer for the five Egyptian cotton varieties based on ISSR analysis

Primer code	Number of amplified bands	DNA length (bp)	Number of Polymorphic bands	Percentage of polymorphism (%)
OPA-01	25	350-2800	5	20
OPA-02	35	380-2500	10	28.5
OPA-03	25	550-2800	4	16
OPA-04	30	420-2800	7	23.3
OPA-05	25	350-3100	7	28
Total	140		33	23.5

Simple Sequence Repeat (SSR) analysis:

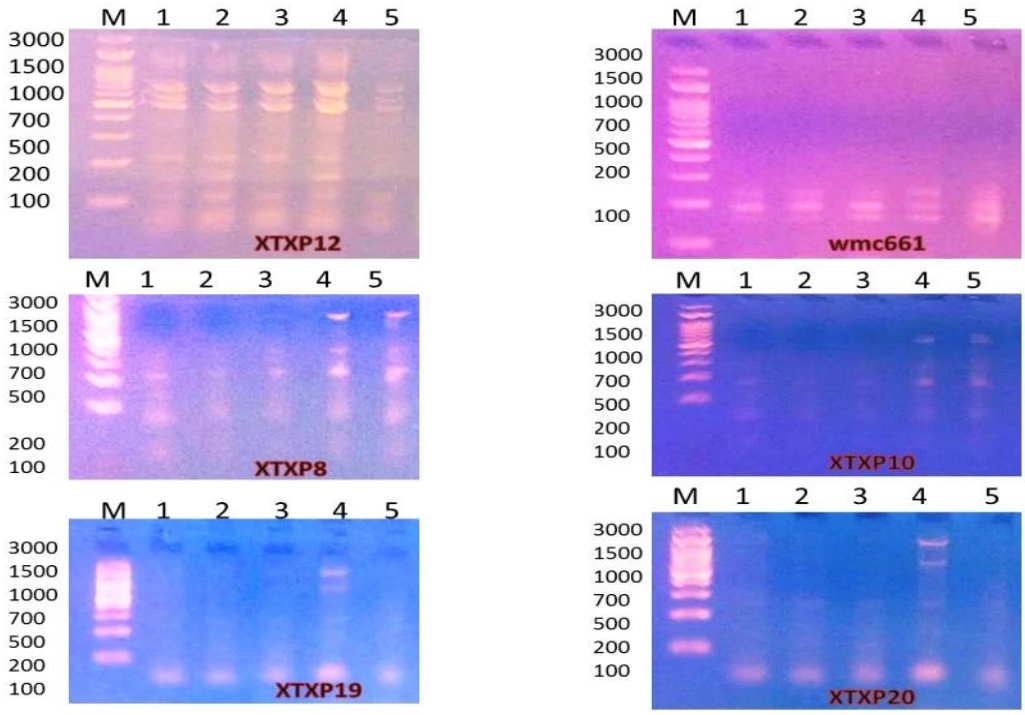

Figure 3. SSR banding profiles of the five Egyptian cotton varieties using six primers of specific sequence repeats (XTXP-12; wmc661; XTXP-8; XTXP-10; XTXP-19 and XTXP-20). M: DNA marker; 1: G92; 2: G93; 3: G86; 4: G87 and 5: G88.

Table 7. The number of amplified and polymorphic fragments and percentages of polymorphism per primer for five Egyptian cotton varieties based on SSR analysis.

Primer code	Number of amplified bands	DNA length (bp)	Number of polymorphic bands	Percentage of polymorphism (%)
XTXP12	35	50-2000	10	28.5
Wmc661	15	150-500	3	20
XTXP8	20	100-2000	7	35
XTXP10	20	200-1500	7	35
XTXP19	20	150-1500	11	55
XTXP20	20	60-2000	12	60
Total	130		50	38

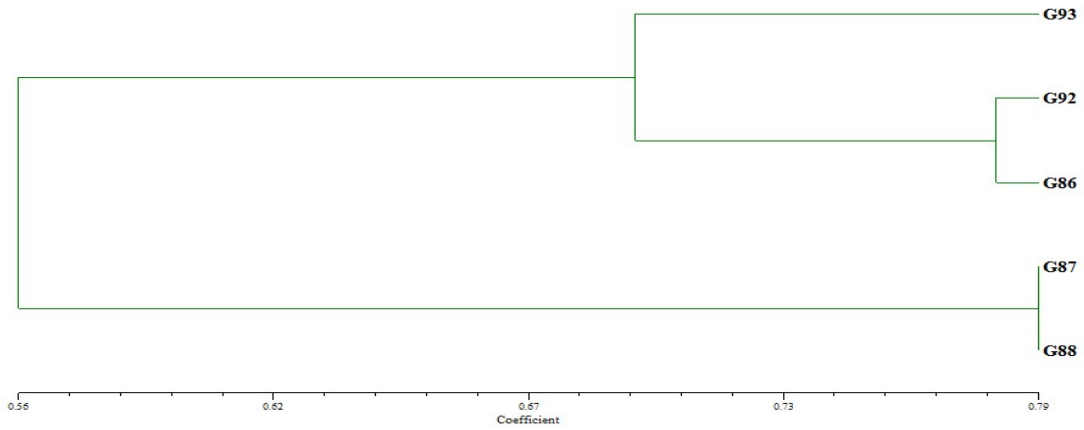

Figure 4.The polygenic relationship of the five Egyptian cotton varieties based on (RAPD+ISSR+SSR)

DISCUSSION

Since the Egyptian government is projected to elevate lint cotton production by increasing the total cultivated area and crop yield, which is associated with improving fiber quality and enhance biotic as well as abiotic tolerance (USDA, 2016). Therefore, it is very important to study the genetic diversity of Egyptian present cotton cultivars, which will be used in breeding programs for the development of new varieties and accessions (Abdel-Fattah, 2010). For this objective, attempts were made in the present investigation to characterize five Egyptian cotton varieties using some physical properties of cotton fibers as well as some DNA molecular markers.

The HVI instrument was mostly applied (Manandhar, 2013; ELÇ, *et al*., 2014; McCormick, 2015), and considered as main tool for cotton breeders to analyze fiber properties. According to Lacape *et al*. (2010), different physical characteristics of cotton fibers are measured ranging from fiber length and length uniformity such as strength; elongation; maturity; micronaire; fineness to color indices. Thus, in the present study, some of these physical properties (Length; micronaire and strength) were determined for the five different cotton fibers using HVI. Depending on the fiber length classification of the international cotton association–BREMEN, our Egyptian cotton cultivars were classified as long (G92; G86 and G88) and extra-long (G93 and G87) staple cotton. Fiber length is largely determined by variety and affects yarn strength; yarn evenness; efficiency of spinning process and fineness (USDA, 2001). Our micronaire reading ranged from fine for the long staple cotton varieties (G92; G86 and G88) to extra-fine for the extra-long staple cotton cultivars (G93 and G87). These results are in agreement with the classification of cotton-USDA-feb2000, which reported that longer fiber is always finer fiber. In addition, strength measurements of the five Egyptian cotton varieties were determined as very strong fibers. Similar data was reported by EL Messiry *et al.* (2012) for G86; G87 and G88 varieties.

In this work, three PCR based markers (RAPD; ISSR and SSR) were applied to determine the genetic diversity and relationships among the five Egyptian cotton varieties. The highest number of the polymorphic fragments and percentage of polymorphism were reported for RAPD. In contrast, the least values were indicated for ISSR markers. SSR analysis revealed intermediate number of polymorphic amplicons and percentage of polymorphism. The overall finding from our data indicated that the three analyses sufficiently detected genetic diversity to differentiate the five Egyptian cotton varieties. The same conclusion was reported for different cotton genotypes after applying different DNA molecular markers (Jing *et al*., 2000; Hussein *et al.,* 2002 & 2006 & 2007; El- Defrawy *et al.,* 2004; Rana and Bhat, 2005; Esmail *et al.,* 2008; Zahid *et al.,* 2009). While, in the present study, RAPD analysis was more efficient than SSR and ISSR markers. In general, the variation in the number of amplicons by different primers influenced by variable factors such as primer structure; template quantity and number of annealing sites in the genome (Kernodle *et al.* 1993). *Gossypium barbadense* has limited genetic diversity. Abdellatif *et al.* (2012) indicated that the ancestors of all the Egyptian cotton cultivars bred in Egypt are limited to 4 varieties only (Ashmoni; Giza 12; Sakha 3 and Sakha 4) which confirms the narrow genetic background of the Egyptian cotton varieties. Therefore, RAPD analysis may offer a powerful tool for analyzing the genetic variability and relationships of cotton genotypes (El-Zanaty *et al*., 2011) because it mostly produce a

large number of amplified fragments comparing with both SSR and ISSR markers (Kahodariya *et al*., 2015).

In the present study, UPGMA analyses were performed, and dendrograms were constructed. Based on RAPD; ISSR; SSR and combined data, two similar clusters with variable percentages of similarity were detected. The first cluster grouped G87 (G77 X G45A) as extra-long extra-fine variety with G88 (G77 X G45B) as long fine cultivar. This phylogenetic relationship may be related to their common ancestors, and also may explain why both varieties are related to each other despite their cotton fibers differences (Hussein *et al.,* 2007). The second cluster included G93; G92 and G86 cultivars. For each DNA molecular data as well as all molecular data combined, the most related varieties were G92 and G86. This finding is in agreement with those obtained by Abdellatef *et al*. (2012). They analyzed 28 Egyptian cotton genotypes (varieties and hybrids) using different molecular markers (RAPD; SSR and EST). According to their cluster of all molecular data combined, they reported that G92 and G86 varieties are genetically closely related, and both of them in the same sub-cluster with Pima S6 as well as the same group with G93 variety (G77 X Pima S6). Although there is no clustering according to the pedigree history for G93 (G77 X Pima S6); G92 {G84 X (G74 X G68)} and G86 (G75 X G81) varieties and also in the cotton fiber properties as extra-long extra-fine (G93) and long fine (G92 and G86), DNA markers could better reveal the genotypic relationships when there are sufficient markers and they are distributed across all chromosomes (Zhang *et al*., 2005).

In conclusion, according to the high economic importance of the Egyptian cotton, considerable attention must be paid for improving cotton plants through breeding programs. The correlation between cotton fiber properties and DNA molecular markers (as rapid and accurate methods) facilitate the classification and the identification of cotton genotypes. In another aspect of the present study, using several DNA molecular marker types of different nature provides a better overall view of differentiation for cotton genotypes as well as evaluation of their genetic polymorphism and relationships. This could be a useful guide for selecting specific germplasm with distinct genetic backgrounds in cotton breeding programs for developing superior cultivars. Finally, further studies are needed to identify a connection among the phenotype and the genotype of the variety-specific markers that were detected in the current investigation.

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الملخص العربي

التوصيف الجزيئي لبعض أصناف القطن المصري

إسراء سيف، محمد الصيحي، سناء رياض، محمود الباجوري

تنتمي شجيرة القطن الى جنس Gossipium والذى يستوطن المناطق الأستوائية والشبه إستوائية وتحتوي لوزة القطن علي ألياف السليلوز النقى، وتعتمد طول ونعومة الألياف على نوع القطن وتقاس بوحدات deatex والتي تتراوح بين ١،١– ٢،٣ decitex يتبع القطن اربع اصناف تجارية: Gossypium hirsutum, Gossypium barbadense, Gossypium arboretum, Gossypium herbaceum كمايتضمن جنس Gossypium ٤٥ نوع ثنائي المجموعة الكرموسومية والتى بها ٢٦ كروموسوم، الى جانب ٥ انواع للمجموعة الكروموسومية المتضاعفة للهجين الرباعى والتى بها ٥٢ كروموسوم

تم دراسة خمس طرز وراثية لأصناف قطن مصرية تتبع Gossypium barbadense (G92, G93, G86, G87 and G88) وقد واضحت النتائج طبقا لجهاز HVI ان متوسط اطوال الالياف بلغت 32.45 مم للاصناف الطويلة (G86) بيمنا للاصناف فائقة الطول فقد كان متوسط اطوالها ٣٣، ٣٥,٣، ٣٥,٢٣ و ٣٢,٩٤ مم (G92, G93,G87، G88) في حين بلغت متوسط قراءة الميكرونير(المؤشر لدرجة النعومة) ٤,٣٩ للاصناف الناعمة (G86) وبالنسبة

للاصناف فائقة النعومة بلغت متوسط قرائتها ٣,٥٩، ٢,٧٧، ٣,١٦، ٣,٩٩ (G92, G93, G87,G88) بينمابلغت متوسط المتانة للاصناف تحت الدراسة 45.85, 41.95, 43.95, 43.00 and 39.99 جم/ تكس (G92, G93, G86, G87 and G88) مما يؤكد المتانة العالية للخمسة اصناف.

وعلى صعيد التحليل الجزيئي للخمسة اصناف تم استخلاص ال DNA وإجراء ال PCR تفاعل السلسلة عديد البوليمر، حيث تم أستخدام خمس بادئات عشوائية فى حالة تكنيك RAPD- PCR والتى قد نتج عنه ١٩٠ حزمة تراواحت اطوالها الجزيئية بين ٢٠٠ – ٢٧٠٠ bp وبلغت نسبة تعدد الاشكال المظهرية حوالي ٤٠% لاجمالى ٧٦ قطعة. بينما قد تم استخدام خمس بادئات فى حالة تكنيك ISSR وقد نتج عن ذلك ١٤٠ حزمة تراوحت اطوالها الجزيئية بين ٣٥٠– ٣١٠٠bp وبلغت نسبة تعدد الاشكال المظهرية حوالى ٢٣,٥% لاجمالي ٣٣ قطعة. فى حين استخدام ست بادئات لتكنيك SSR والتى نتج عنها ١٣٠ حزمة تراوحت اطوالها الجزيئية بين ٦٠ –٢٠٠٠bp وبلغت نسبة تعدد الاشكال المظهرية حوالى ٣٨% لاجمالي ٥٠ قطعة. بالإضافة الي ذلك فقد اوضحت الدراسة الحالية من خلال استخدام ثلاث تكنيكات الواسمات الجزيئية RAPD, ISSR and SSR والتى كانت قادرة على التفريق بين اربعة اصناف من (G92, G93, G87 and G88) من اصل خمس اصناف قطن مصري تحت الدراسة G92, G93, G86, G87 and G88) وذلك من خلال الكشف عن ١٧ واسمة جزيئية خاصة بالصنف والتى يمكن استخدامها للتفرقة فى بين جميع الاصناف تحت الدراسة ماعدا G86 وبناءا على هذا جاء العديد من التقاريرحول استخدام الواسمات الجزيئية لل DNA المختلفة التى تؤكد اهمية الواسمات الخاصة بالصنف و/ النوع فى تعريف نبات القطن الى جانب دراسةعمليات نقل الجين التى تفيد المربي فى نقل الصفات المرغوب فيها من صنف لاخر

طبقا لنتائج الواسمات الجزيئية لتكنيكات ال RAPD, ISSR and SSR فقد اظهرت شجرة النسب عن وجود مجموعتين متشابهتين بنسب متغيرة، حيث تحتوى المجموعة الاولي الاصناف فائقة الطول G87(G77XG45A) و G88(G77XG45B) وترجع هذه العلاقة الى وجود اباء مشتركين بينهم مما يوضح ارتباط هذين الصنفين ببعضهما البعض على الرغم الاختلاف الملحوظ فى الصفات المظهرية للالياف لكل منهما. بينما تحتوى المجموعة الثانية على الاصناف

G92(Giza84 x (Giza74 و G93 (Giza77 X Pima S6) x Giza 68), G86(Giza 75 x Giza 81)).

ويستنتج من هذه الدراسة ان لابد من اهتمام الدولة بتحسين القطن المصري كونه محصول اقتصادى هام وذلك من خلال برامج التربية. حيث الارتباط مابين خواص الياف القطن والواسمات الجزيئية لل DNA كالطرق الدقيقة والسريعة يساعد فى تصنيف وتعريف الطرز الوراثية للقطن. وعلى صعيدا اخر استخدام العديد من الواسمات الجزيئية لل DNA من مصادر مختلفة يمنح دراسة عامة جيدة فى التفرقة للطرز الوراثية للقطن وتقييم العلاقات وتعدد الاشكال المظهرية الوراثية. وهذا يمكن ان يكون مرشدا جيد لعمليات انتخاب محتوي وراثى محدد باصول وراثية مميزة فى برامج تربية نبات القطن لاستنباط اصناف فائقة. واخيرا لابد من اجراء العديد من الدراسات لتحديد شكل العلاقة فيما بين الاشكال المظهرية والطرز الوراثية من خلال الواسمات الوراثية الخاصة بالصنف والتى تم تحديدها فى هذه الدراسة.