

Full Length Research Paper

Genetic relationships of cotton (*Gossypium barbadense* L.) genotypes as studied by morphological and molecular markers

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Fifteen (15) morphological traits and three different types of molecular markers [inter simple sequence repeats (ISSR), simple sequence repeat (SSR) and expressed sequence tag (EST) markers] were used to study the genetic relationships among 24 cotton (*Gossypium barbadense* L.) genotypes (commercial varieties and new germplasm). High significant differences were observed among the genotypes for all the studied traits and the interaction between genotypes and years ranged from highly significant to significant for the most studied traits. The value of phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all studied traits which means that the apparent variation is not only due to genotypes but also due to the influence of environmental factors. The cluster analysis of the 24 cotton genotypes depending upon the morphological traits divided them into two main groups (A and B) while molecular data divided them into six groups. The cotton genotypes were distributed according to principal coordinate analysis (PCORDA) analysis of both morphological traits and molecular markers regardless of their fiber characteristics. According to this analysis, the cotton genotypes were distributed into three distinct parts. Most molecular markers showed polymorphism in their patterns. The highest number of total and polymorphic bands was generated from ISSR markers while the least number of total and polymorphic bands was obtained from the EST-SSR markers. According to both morphological and molecular analyses, the following genotypes could be used to hybridize and produce high growth and yield potential: Giz87, Giza45, Giza88 and Giza70 as a first parent and Karshansky, Giza80, Giza83, Australian10229 and Russian6022 as a second parent in the cross.

Key words: Cotton, simple sequence repeat (SSR), expressed sequence tag (EST), inter simple sequence repeats (ISSR), morphological traits, cluster analysis, principal coordinate analysis (PCORDA).

INTRODUCTION

Genetic diversity and relationship is a raw material for industrial agriculture and to achieve sustainable agriculture because it enables farmers to adopt crops suitable for their own site specific ecological needs and cultural traditions. Genetic relationship enables long term sus-

tainability and agricultural self-reliance and has been known to increase or decrease in response to domestication. 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).

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Although, it is widely assumed that genetically diverse parents facilitate the creation of superior progeny, few studies have examined the relationship between parental genetic distance and the creation of successful varieties. Interspecific and intraspecific hybridization with the cultivated tetraploid germplasm result in high genetic variation and recombination. Linkage drag is a problem in the selection of hybrid combinations of interspecific and intraspecific gene transfer in cotton because of its polyploidy nature. In theory, mating of distantly-related parents will produce a greater number of transgressive segregates than mating of closely-related parents. In this respect, the genetically farthest parents are expected to produce new recombination. Cornelius and Sneller (2002) reported that a lack of genetic relationship may limit breeding progress and gain from selection. Information of genetic relationship is important when working to improve crop and develop new varieties. Also, characterizing genetic relationship and degree of association between and within varieties is the first step toward developing germplasm and crop varieties. Successful crop improvement depends on genetic variability that arises from genetic relationship (Rana and Bhat, 2005).

The best process to assess the genetic relationship is to combine both morphological traits with molecular markers in order to build better genetic figure of the nature of genetic relationship than using one of them. Molecular markers play an important role in study of genetic relationship in crop plants and as well as in cotton. Several types of PCR-based DNA markers have been utilized in cotton genome research, including RAPD, ISSR, SSR, AFLP, RGA and SRAP (Zhang et al., 2008; Abdellatif et al., 2012).

Inter simple sequence repeats (ISSR) is an easy and informative genetic marker system in cotton for revealing both inter and intraspecific variations (Liu and Wendel, 2001). ISSR technique uses primers that are complementary to a single SSR and anchored at either the 5' or 3' end with one- to three-base extension. The amplicons generated consist of regions between neighboring and inverted SSRs (Zietkiewicz et al., 1994; Preetha and Raveendren, 2008).

Simple sequence repeats (SSRs) are considered to be ideal and friendly tools for such studies as they are polymerase chain reaction (PCR)-based markers, genetically defined, typically co-dominant and uniformly dispersed throughout plant genomes (Morgante et al., 2002; Turkoglu et al., 2010). For these reasons, SSRs have become an important marker system in cultivar fingerprinting, relationship research and molecular mapping (Reddy et al., 2001). In addition, SSR markers derived from expressed sequence tags (EST-SSRs) are likely to be even more transferable than genomic SSRs because they are located in the transcribed regions of the genome (Park et al., 2005). Sometimes an EST-SSR

marker may be a part of functional gene itself. These qualities have drawn much more attention to marker-assisted selection and comparative mapping in cotton breeding. The additional studies in this issue are expected to assist in maximizing the selection of diverse parent genotypes and broadening the germplasm base of cotton breeding programs in the future.

The aim of the present investigation was to study genetic relationship of cotton (*Gossypium barbadense*) genotypes using both morphological traits and molecular markers (ISSR, SSR and EST) and collect essential information to produce new recombination that could be used in cotton breeding programs.

MATERIALS AND METHODS

Twenty-four (24) cotton (*G. barbadense* L) genotypes (commercial varieties and new germplasm) were used in this study. Seed material was provided by the Cotton Research Institute, Agricultural Research Center, Egypt (Table 1).

Experimental design and traits measurements

A field experiment was conducted to evaluate the growth, yield components and fiber quality traits performance of cotton genotypes. The field experiment was conducted during the cotton growing seasons of 2011 and 2012 at randomized complete block design (RCBD) with three replications. Each plot consisted of one row 5.5 m in length with 70 cm between rows. Hills were spaced by 40 cm apart and comprised of one plant/hill so as to have 15 plants per row.

Fifteen morphological traits were recorded from 10 randomly selected plants per replication. These traits included first fruiting node (F.FN), days to first flower (D.F.F.), boll weight in gram (BW), seed cotton yield/plant (g) (SCY/P), lint cotton yield/plant (L.C.Y/P.), lint percentage (L.P%), number of open bolls/ plant (No.B/P), seed index in grams (S.I.), number of seeds/boll (S/B), lint index in grams (L.I), 2.5% span length (mm) (F.L), fiber strength (F.S), micronaire reading ($\mu\text{g}/\text{inch}$) (F.F), uniformity ratio (UR) and fiber yellowness (+b). Lint samples were submitted to laboratories of cotton technology department, Cotton Research Institute at Giza, Egypt to tests all fiber properties at constant atmospheric condition of 65 ± 2 R.H and $21 \pm 2^\circ\text{C}$ temperatures.

DNA isolation

Cotton seeds were grown in the green house for ten days; leaves of seedlings were collected and ground 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., 1983).

Molecular markers analyses

Five cotton specific EST-SSR primer pairs, six primer pairs specific for cotton microsatellite (SSR) and five SSR-anchored primers (ISSR primers) were used to perform the molecular analyses (Table 2) according to Abdellatif et al. (2012). The PCR amplification reac-

Table 1. The commercial name of the genetic materials, their background, classification category and growth climatic region.

| Number | Name | Abbreviation | Origin | Category | Region |
|--------|-------------------------------------|----------------------|---|-----------------------------------|-----------|
| 1 | Giza 45 | G.45 | Giza 28xGiza 7 | Extra long staple | Egypt |
| 2 | Giza 70 | G.70 | Giza 59b x Giza 51b | Extra long staple | Egypt |
| 3 | Giza 87 | G.87 | (Giza 77 x Giza 45)a | Extra long staple | Egypt |
| 4 | Giza 92 | G.92 | Giza 84 x (Giza 74 x Giza 68) | Extra long staple | Egypt |
| 5 | Giza 84 | G.84 | Giza 68 x C.B 58 | Extra long staple | Egypt |
| 6 | Giza 77 x PS 6 (creamy) | G.77*PS6 C | Giza 77 x PS ₆ C | Promising Extra long staple cross | Egypt |
| 7 | Giza 77 x PS 6 (White) | G.77*PS6 W | Giza 77 x PS ₆ W | Extra long staple | Egypt |
| 8 | Giza 86 | G.86 | Giza 75 x Giza 81 | long Staple | Egypt |
| 9 | Estroly 13 | Estr.13 | - | long Staple | Australia |
| 10 | Pima high percentage | Pima_HP | - | long Staple | US-Egypt |
| 11 | Giza 88 | G.88 | (Giza 77 x Giza 45)b | Extra long staple | Egypt |
| 12 | Giza 84 x Giza 70 x Giza 51b x P 62 | G84*G70 | [Giza 84 x (Giza 70 x Giza 51b)] x P 62 | Promising Extra long staple cross | Egypt |
| 13 | Pima 62 | P. 62 | - | long Staple | US-Egypt |
| 14 | Giza 89 | G. 89 | Giza 75 x Russ.6022 | long Staple | Egypt |
| 15 | Giza 89 x Giza 86 | G.89*G.86 | Giza 89 x Giza 86 | long Staple | Egypt |
| 16 | Giza 75 x Sea | G.75*S | Giza 75 x Sea | long Staple | Egypt |
| 17 | Giza 89 x PS 6 | G.89*PS ₆ | Giza 89 x PS ₆ | long Staple | Egypt |
| 18 | Karshanese branches | Karsh. | - | long Staple | Russia |
| 19 | Suvin | Suvin | Sujata x Vincent | long Staple | India |
| 20 | Giza 83 | G.83 | Giza 72 x Giza 67 | long Staple | Egypt |
| 21 | Pima S6 | P.S ₆ | - | long Staple | US-Egypt |
| 22 | Russian 6022 | Russ. 6022 | - | long Staple | Russia |
| 23 | Giza 80 | G.80 | Giza 66 x Giza 73 | long Staple | Egypt |
| 24 | Australian 10229 | Aust.10229 | - | long Staple | Australia |

tions were achieved in a 25- μ l volume using 50 ng DNA containing 0.3 μ moles of each primer, 200 μ M of dNTPs, 5 μ l (1X) of Taq polymerase buffer, 2 mM MgCl₂ and 0.5 U Taq DNA polymerase. The reactions of each marker type were carried out according to Abdellatif et al. (2012). PCR products were separated on 1.5% agarose gel electrophoresis.

Statistical analysis

The mean values of the 15 morphological traits measured in the 24 genotypes in each replication over all two years were analyzed to estimate analysis of variance over all years and heritability in broad sense by Milligan et al. (2003). Phenotypic, genotypic and environmental coefficient variability were calculated according to Singh and Narayanan (2000). The data on the 15 morphological traits were subjected to multivariate hierarchical cluster analysis using NTSYSPC v2.1 software (Rohlf, 1998). Principal coordinate analysis (PCOORDA) was also performed on the basis of the distance matrices, using the standardized centered data in the NTSYS PC2.1 software. Similarities matrices were calculated using SimInt option, decentered, and then the eigenvectors and the eigenvalues were calculated using the ordination option in NTSYS PC program then the two-dimensional diagram was obtained.

All gels of the different molecular markers were scored as 0/1 for absence/presence of the bands, respectively. The total number of band and the number of polymorphic bands were calculated as well

as the polymorphic information content (PIC) which was calculated according to Anderson et al. (1993). Similarity coefficient matrices were calculated for all the markers (mixed together) using simple matching similarity algorithm (Sokal and Sneath, 1963). Phylogenetic dendrogram was constructed using the UPGMA method (Sneath and Sokal, 1973). The molecular data were standardized through NTSYS PC2.1 software and then principal coordinate analysis was conducted as illustrated for the morphological traits.

RESULTS AND DISCUSSION

Morphological traits

The combined (throughout the the two years) analysis of variance (ANOVA) of the 24 cotton genotypes showed high significant differences among the genotypes through all the studied traits indicating the existence of relatively large genetic variability among the studied traits and the genetic materials in the present investigation differed widely in their performances (Table 2). The differences due to the interaction among genotypes and years ranged from high significant to significant for the most studied traits, while it was not significant for the traits

Table 2. Mean square obtained from combined analysis of variance over two years of 24 cotton genotypes, broad sense heritability (H), and phenotypic (PCV%), genotypic (GCV%) and environmental (ECV%) coefficient of variability for all morphological traits.

| S.O.V | df | FFN | DFF | BW | SCY | LCY | LP | No.B./p | SI | SB | Li | FL | FF | FS | UR | +b |
|-----------|----|----------|---------|---------|------------|------------|---------|----------|--------|--------|---------|---------|--------|--------|----------|--------|
| Years | 1 | 46.376** | 8.042 | 0.212* | 16148.90** | 66292.52** | 51.58** | 2555.38* | 8.54** | 18.69* | 12.61** | 4.45 | 0.61** | 9.61** | 0.01 | 0.001 |
| Error a | 4 | 0.806 | 4.127 | 0.016 | 1042.06 | 78.74 | 1.41 | 162.31 | 0.18 | 1.75 | 0.13 | 0.717 | 0.03 | 0.07 | 0.85 | 0.152 |
| Genotypes | 23 | 2.675** | 40.01** | 0.178** | 5833.98** | 503.11** | 8.52** | 751.13** | 0.69** | 6.79** | 0.81** | 19.98** | 1.49** | 2.66** | 12.694** | 7.77** |
| G*Y | 23 | 0.246 | 4.183* | 0.032 | 3533.01** | 385.45** | 2.54** | 475.37** | 0.56** | 3.66** | 0.45** | 1.07** | 0.043 | 0.33** | 2.39* | 0.03 |
| Error b | 92 | 0.21 | 2.31 | 0.02 | 809.85 | 105.77 | 1.21 | 82.96 | 0.19 | 1.31 | 0.14 | 0.48 | 0.04 | 0.14 | 1.30 | 0.04 |
| H | | 54.30 | 58.72 | 41.11 | 27.52 | 20.99 | 34.84 | 29.04 | 18.14 | 25.34 | 25.90 | 76.29 | 77.93 | 58.92 | 44.01 | 95.00 |
| GCV (%) | | 8.26 | 3.29 | 5.81 | 17.16 | 19.03 | 3.14 | 17.84 | 3.14 | 5.51 | 5.91 | 5.41 | 12.22 | 6.49 | 1.66 | 11.60 |
| PCV (%) | | 11.21 | 4.29 | 9.06 | 32.72 | 41.55 | 5.32 | 33.11 | 7.38 | 10.94 | 11.62 | 6.19 | 13.84 | 8.45 | 2.50 | 11.90 |
| ECV (%) | | 6.95 | 2.39 | 6.22 | 20.23 | 28.03 | 3.64 | 18.81 | 5.31 | 7.60 | 7.85 | 2.53 | 6.02 | 4.49 | 1.62 | 2.59 |

FFN, BW, FF and +b (Table 2). High broad sense heritability estimates were detected for all fiber properties traits (FL, FF, FS, UR, +b) and earliness traits (FFN and DFF) indicating that these traits could be improved through pure line selection. Our results agreed with those found by Hamoued and Yehia (2009) and Esmail et al. (2007). While, moderate and low detected for yield and its components traits indicating that environments factors play the greater role in these traits. Hendawy (1994) found relatively low heritability value for seed cotton yield in two cotton crosses (46.32 and 49.42%). Also, low heritability with low genetic gain was found for seed cotton yield per plant in the *barbadense* cross (2.27 and 1.7), indicating slow progress through selection for this trait. However, Mahros (2008) found moderate to high broad sense heritability estimates for seed cotton yield and most of its components in the three cotton crosses.

The value of phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all studied traits which means that the apparent variation is not only due to

genotypes but also due to the influence of environmental factors. On this base, such traits are highly influenced by the environmental conditions. Selection for such traits sometimes may be misleading (Singh and Narayanan, 2000). Abd-El-Haleem et al. (2010) found similar results when they studied the genetic analysis of yield and its components in seven Egyptian cotton varieties. They reported that the phenotypic coefficient (PCV) of variability values were higher than GCV in the four crosses for all traits except for days to 50% maturity trait, indicating that these traits are more sensitive to the environmental conditions. Esmail et al. (2008) reported that high broad sense heritability estimates were detected for all traits studied when they used morphological traits to study the genetic relationships of 21 cotton genotypes.

Morphological cluster analysis

The cluster analysis of the 24 cotton genotypes depending upon the morphological traits divided

them into two main groups (A and B). Group A was divided into two subgroups. Subgroup A1 included six genotypes namely, Giza70, Giza87, Estroly13, G77XPS6 (creamy), Pima S6 high percentage and the accession Australian 10229. The two most related genotypes through this subgroup were Giza70 and Giza87 varieties (Giza70 is an ancestor parent of Giza87) (Abdel-Salam, 1999) followed by PimaS6-high percentage and the accession Australian10229. The subgroup (A2) contained the genotypes Giza92 and the hybrid G77XPS6 (white) (Giza68 variety is a common ancestor of both varieties) while Giza 45 variety separated these two subgroups (Figure 1). The later is different from the other varieties in its performance and genetic background. The second group (Group B) also was divided into two subgroups. Subgroup B1 was divided into two sub-subgroups, sub-subgroup B11 including six genotypes (Giza84, Giza88, G89XG86, Giza83, Giza80 and Giza89 genotypes) and sub-subgroup B12 including seven genotypes (Giza86, G84XG70XG51bXP62, PimaS6, Pima62, Suven, Karshansky branches

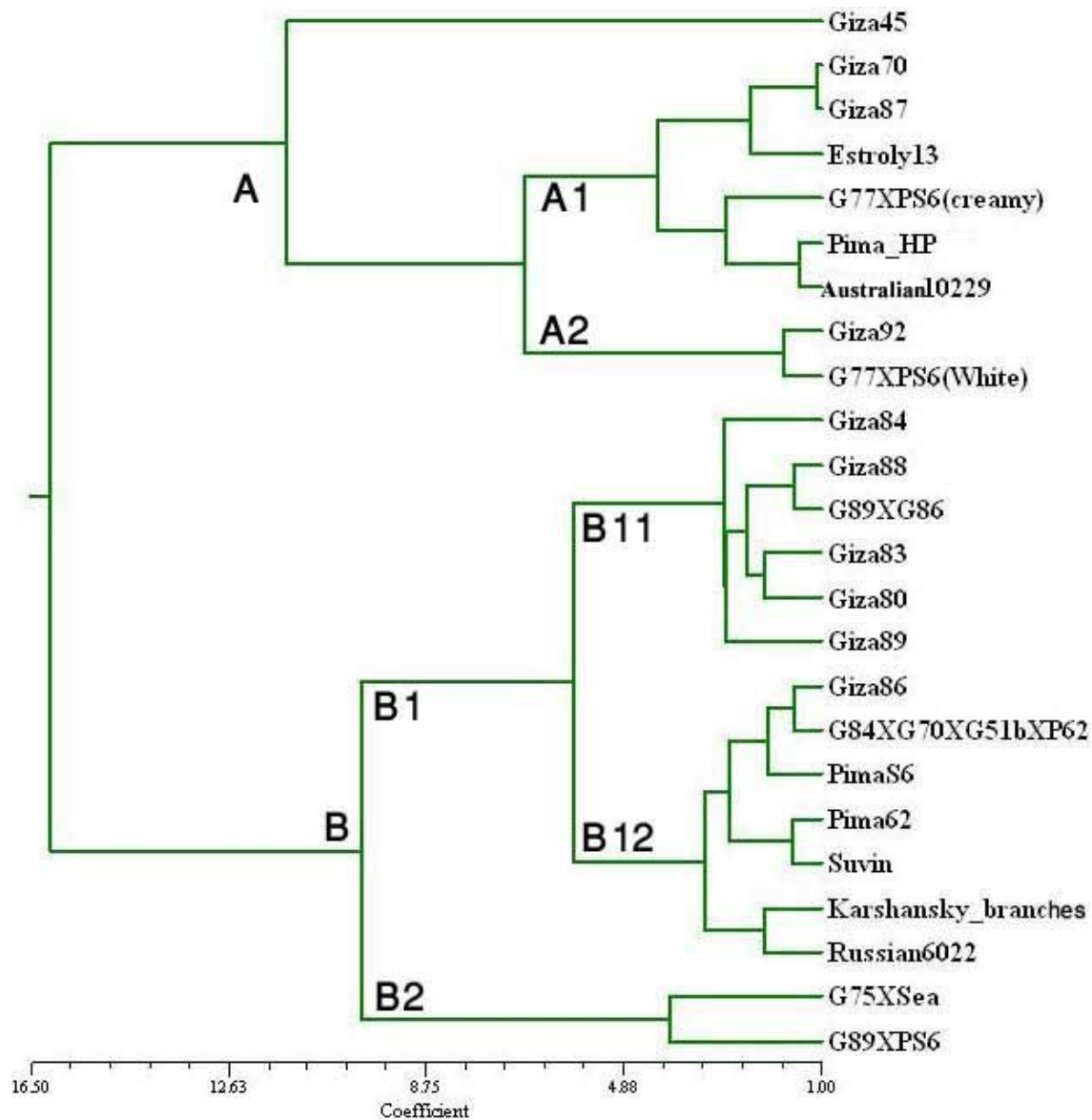


Figure 1. Dendrogram of 24 cotton genotypes constructed depending upon morphological traits using Euclidean's distance coefficient and UPGMA method.

and Russian6022). The most related genotypes in this subgroup were Giza88 and the hybrid G89XG86 genotypes was followed by Giza86 and the promising cross G84XG70XG51bXP62 genotypes (Giza36 variety is a common ancestor for the four genotypes) (Abdel-Salam, 1999) (Figure 1). The subgroup B2 contained only two genotypes G75XSea and G89XPS6 genotypes (Figure 1). These two genotypes share common parent (Giza75).

According to this analysis, any member of group A could be used to hybridize with any member from Group B. The best produced cross with high variability and new recombination depends on the genetic distance. So, the

genetic distance among the varieties included in the hybrid, good and high growth vigor hybrid will be obtained. Zhang et al. (2011) assessed the genetic diversity of cotton cultivars using genomic and noticed a relatively high level of genetic variation depending upon the produced dendrograms.

Esmail et al. (2008) obtained large variability for yield and its components between cotton genotypes. They reported that the cluster analysis based on Euclidean distance using yield characters grouped the 21 cotton genotypes into two main groups at 20 Euclidean distances. Cluster "A" and "B" composed of 11 and 10 genotypes, respectively.

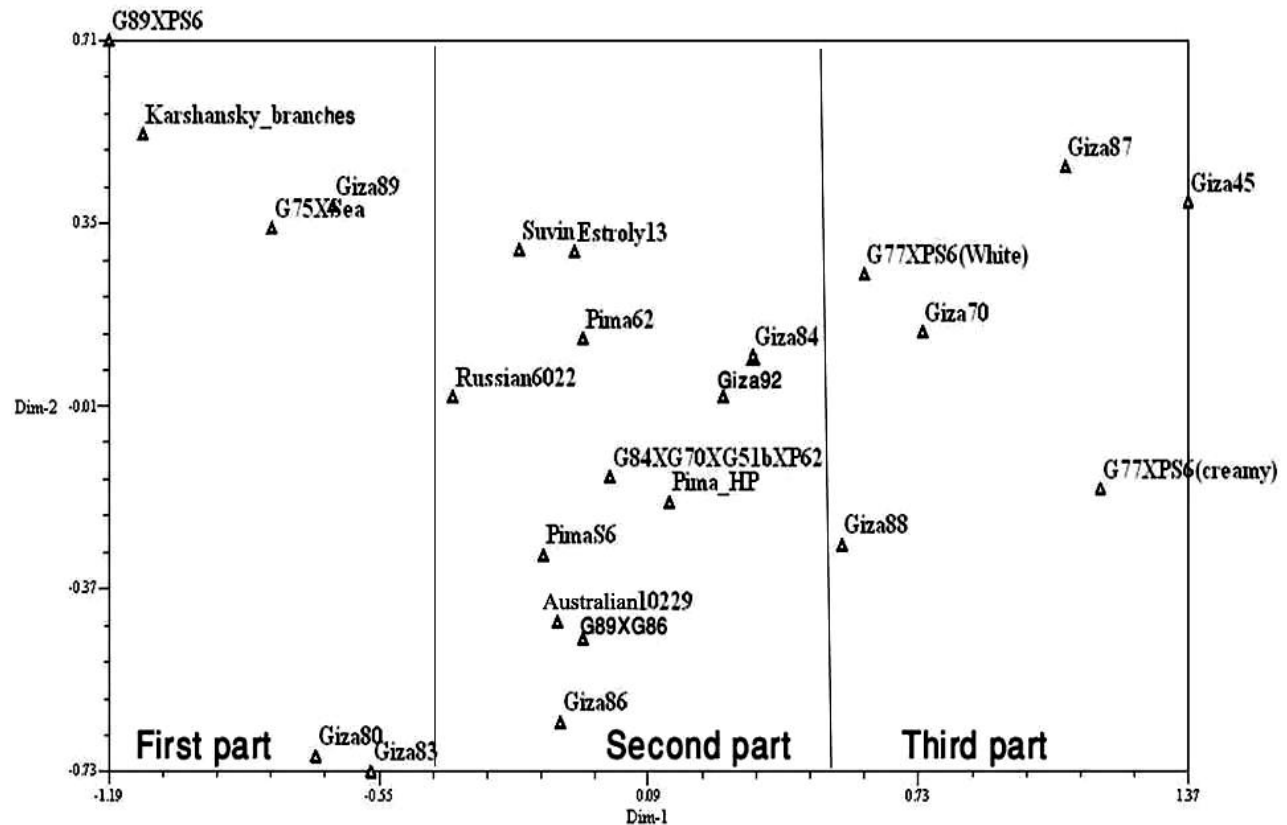


Figure 2. Two-dimensional plot of principal coordinate analysis of 24 cotton genotype based on morphological traits (PC1 = 45.8%, PC2 = 16.3%).

Morphological principal coordinate analysis (PCOORDA)

The first two principal coordinates represent about 62% of the genetic variation found in the cotton genotypes (45.8% for PC1 and 16.3% for PC2 (Figure 2). This means that PCOORDA analysis could be used efficiently to study the genetic relationship among the cotton genotypes under study according to Mohammadi and Prasanna (2003).

They reported that principal coordinate analysis (the ordination method) can be used for genetic diversity determination purposes, particularly when the first two or three PCs explain more than 25% of the genetic variation. The cotton genotypes were distributed according to PCOORDA analysis of morphological traits regardless of their fiber characteristics.

The first principal coordinate for the morphological traits represents almost half of the genetic variance (45.8%, Figure 2), so it could be used to discuss the genetic relationships among the cotton genotypes. According to this analysis, the cotton genotypes was divided into three parts, the first part included G89XPS6, Karshansky

branches, Giza89, G75XSea, Giza80 and Giza83 genotypes (represents the long staple cotton genotypes). The second part (represents both long and extra long staple cotton genotypes) containing the genotypes Suvin, Estroly13, Pima62, Giza84, Giza92, Russian6022, G84XG70XG51bXP62 (promising cross), Pima_HP, PimaS6, Australian10229, G89XG86 and Giza86 (Figure 2). The third part (represents the extra long staple cotton genotypes) included Giza87, Giza45, G77XPS6 (white), Giza70, G77XPS6 (creamy) and Giza88 genotypes. It would be expected that the genotypes included in each part having stronger genetic relationships among each other was compared with the genetic relationships among genotypes from different parts in this analysis. By this way, it could be concluded that a successive cross with new recombination in cotton breeding programs could be obtained by using genotypes from the first part to be mated with genotypes from the third part. El-Mansy et al. (2012) studied the genetic diversity and its relation with cotton varietal development. They revealed that principal component analysis is an efficient analysis to determine the varietal development and to study the genetic diversity in cotton.

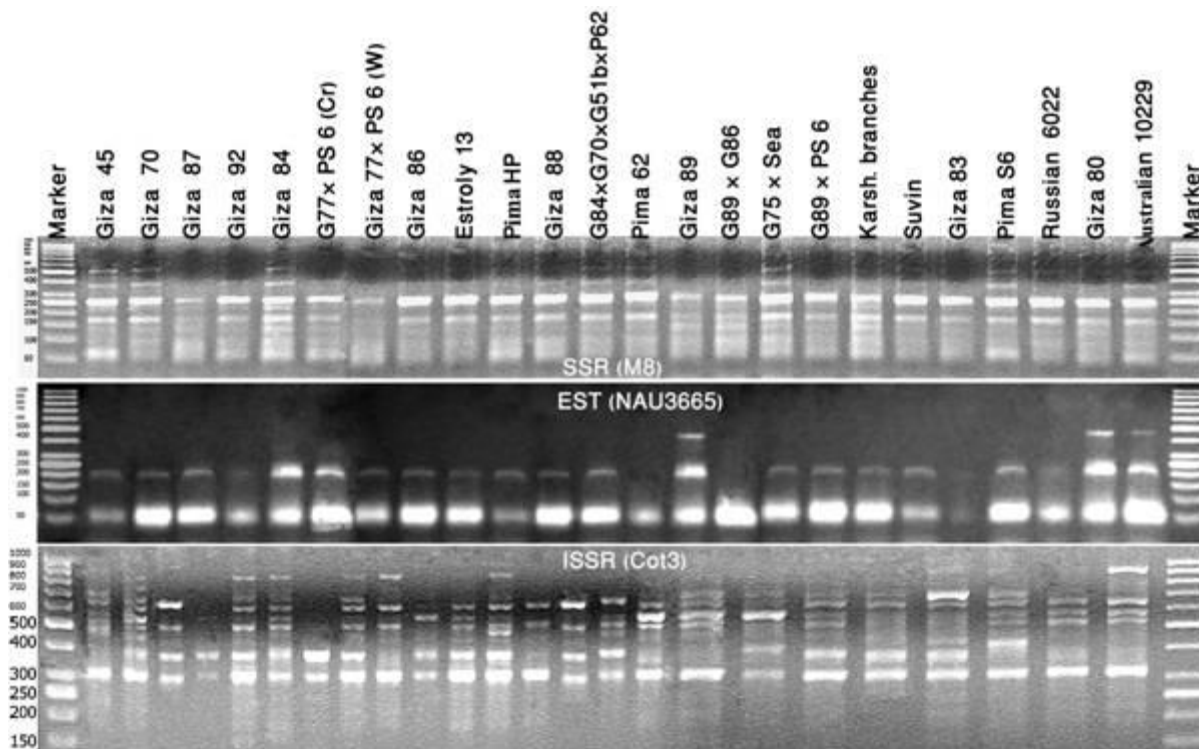


Figure 3. PCR products patterns of 24 cotton genotypes separated on 1.5% agarose gel electrophoresis using three different types of molecular markers.

Molecular markers

Three different types of molecular markers (SSR, EST-SSR and ISSR markers) were used to study the genetic relationships of 24 cotton (*G. barbadense*) genotypes. Most markers showed polymorphism in their patterns. The highest number of total and polymorphic bands was generated from ISSR markers while the least number of total and polymorphic bands was obtained from the EST-SSR markers (Figure 3).

The total number of bands of ISSR primers ranged from seven (for primers Cot 1 and Cot 4) to 21 (for the primer Cot 3) while the polymorphic bands ranged from six bands (for the primer Cot1) to 20 (for the primer Cot 3). The percentage of ISSR primers polymorphism ranged from 85.7% (for the primer Cot 1) to 100% (for the primers Cot 4 and Cot 5) while the polymorphic information content (PIC) ranged from 0.87 (for primers Cot 1 and Cot 4) to 0.96 (for the primer Cot 3, Table 3).

The total number of bands of SSR primers ranged from four (for primer pair M11) to ten (for the primer pair C2-0109) while the polymorphic bands ranged from three bands (for the primer pair M11) to eight (for the primer pairs C2-0109, C2-0119 and SSR3). The percentage of SSR primers polymorphism ranged from 66.7 (for the primer pair M8) to 100% (for the primer pair C2-0119)

while the polymorphic information content (PIC) ranged from 0.75 (for primer pair M11) to 0.90 (for the primer pair SSR3, Table 3).

The total number of bands of EST-SSR primer pairs ranged from three (for primer pair NAU3442) to six (for the primer pairs NAU3665 and E10) while the number of polymorphic bands ranged from two bands (for the primer pairs NAU3442 and C3) to five (for the primer pair NAU3665). The percentage of EST-SSR primers polymorphism ranged from 50 (for the primer pair C3) to 83.3% (for the primer pairs NAU3665 and E10) while PIC ranged from 0.62 (for primer pair C3) to 0.85 (for the primer pairs NAU3665 and E10, Table 3).

Molecular cluster analysis

Cluster analysis of molecular data divided the cotton genotypes into six groups. Group A contained four genotypes namely Giza45, Giza70, Giza86 and Estroly13 while group B contained the genotypes Giza87, Giza92, G77XPS6 (creamy) and Giza84 (Figure 4). The genotypes included in the two above mentioned groups are extra long staple genotypes except Giza86 and Estroly13 genotypes. The third group (group C) was divided into two subgroups, subgroup C1 included four

Table 3. Primer names, number of amplified and polymorphic bands and the polymorphic information content (PIC) generated by EST, SSR and ISSR markers in cotton genotypes.

| Primer name | Bands number | Polymorphic band | Polymorphic (%) | PIC |
|---------------------|--------------|------------------|-----------------|------|
| ISSR primers | | | | |
| Cot 1 | 7 | 6 | 85.7 | 0.87 |
| Cot 2 | 8 | 7 | 87.5 | 0.89 |
| Cot 3 | 21 | 20 | 95.2 | 0.96 |
| Cot 4 | 7 | 7 | 100 | 0.87 |
| Cot 5 | 13 | 13 | 100 | 0.91 |
| SSR Primers | | | | |
| L11 | 8 | 6 | 75 | 0.85 |
| M8 | 9 | 6 | 66.7 | 0.82 |
| M11 | 4 | 3 | 75 | 0.75 |
| C2-0109 | 10 | 8 | 80 | 0.88 |
| C2-0119 | 8 | 8 | 100 | 0.85 |
| SSR3 | 9 | 8 | 88.9 | 0.90 |
| EST Primers | | | | |
| NAU3442 | 3 | 2 | 66.7 | 0.68 |
| NAU3401 | 4 | 3 | 75 | 0.75 |
| NAU3665 | 6 | 5 | 83.3 | 0.85 |
| C3 | 4 | 2 | 50 | 0.62 |
| E10 | 6 | 5 | 83.3 | 0.85 |

genotypes (Giza88, G84XG70XG51bXP62, Giza89 and G89XG86) while subgroup C2 included three genotypes (Pima62, G75XSea and G89XPS6). The genotypes Giza88, G84XG70XG51bXP62 are extra long staple genotypes and have almost similar characteristics except of the yellowness trait (+b). The genotype G84XG70XG51bXP62 (promising cross) is expected to be released as a commercial variety instead of Giza88 variety.

The other two genotypes in subgroup C1 (Giza89 and G89XG86) have similar characteristics and represent the long staple genotypes. Group D included two genotypes (G77XPS6 (white) and Pima_HP). The genotypes PS6 and Pima_HP have the same genetic background and they were almost produced from the old Egyptian cotton variety Meet Afify. The group E included three genotypes (Karshansky_branches, Giza83 and Suvin). All these genotypes are characterized by heat stress tolerance (data not shown here).

The last group (group F) contained the genotypes PimaS6, Russian6022, Giza80 and Australian10229 genotypes (Figure 4). Abdellatif et al. (2012) found the same results concerning the genetic relationships among cotton genotypes. They reported that molecular markers could be used efficiently to study genetic diversity in cotton genotypes.

Molecular PCOORDA analysis

The two dimensional plot of principal coordinate analysis of molecular data revealed that the first three coordinates compromised 27.5% of the total genetic variation in the genotypes. In this case, PCOORDA analysis could be used to analyze the genetic relationships among cotton genotypes. The first two principal coordinates (PCs) representing 23.4% of the total genetic variance. The most important PC was PC1 which represents 11.8% of the total genetic variance among the 24 cotton genotypes. The cotton genotypes were distributed depending upon the later into three parts (Figure 5). The first part included seven genotypes (Australian10229, Russian6022, Giza80, PimaS6, Suvin, Giza84 and Karshansky_branches genotypes) while part two contained five genotypes (G77XSea, G89XPS6, Pima62, Giza89 and G89XG86 genotypes, Figure 5). The other twelve genotypes were aligned in the third part of the PCOORDA analysis depending upon the first PC. The genotypes included in each part are proposed to have high genetic relationships among them as compared to the genotypes included in the other parts. By this way, it could be speculated that hybridization of genotypes from the first part with genotypes from the third part could produce genotypes with high growth and yield potential.

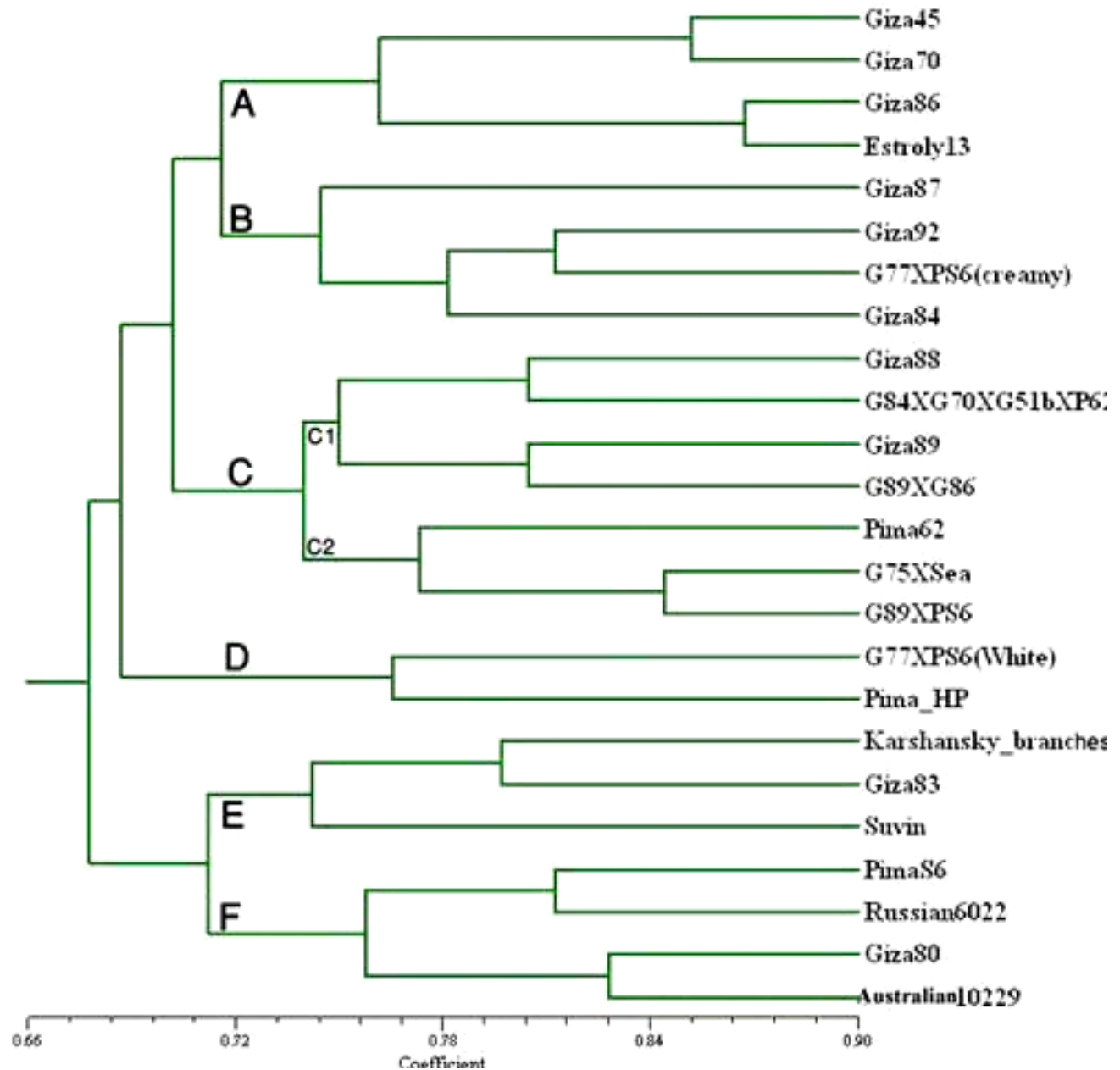


Figure 4. Dendrogram of 24 cotton genotypes constructed depending upon molecular markers using simple matching similarity coefficient and UPGMA method.

Conclusion

According to both morphological and molecular analyses, the following genotypes could be used to hybridize and produce high growth and yield potential: Giza87, Giza45, Giza88 and Giza70 as first parent and Karshansky, Giza80, Giza83, Australian10229 and Russian6022 as a second parent in the cross. The above mentioned genotypes are far from each other according to the

principal coordinate analysis and thus it is expected that their hybridization could aggregate different genetic material from different resources which enrich the genetic diversity and magnitude of the new recombination. Thus, further studies on this point are required to ensure that the above mentioned speculation is correct. Comparing both dominant and co-dominant molecular markers in this study, ISSR markers were most informative than the other molecular markers while the co-dominant markers

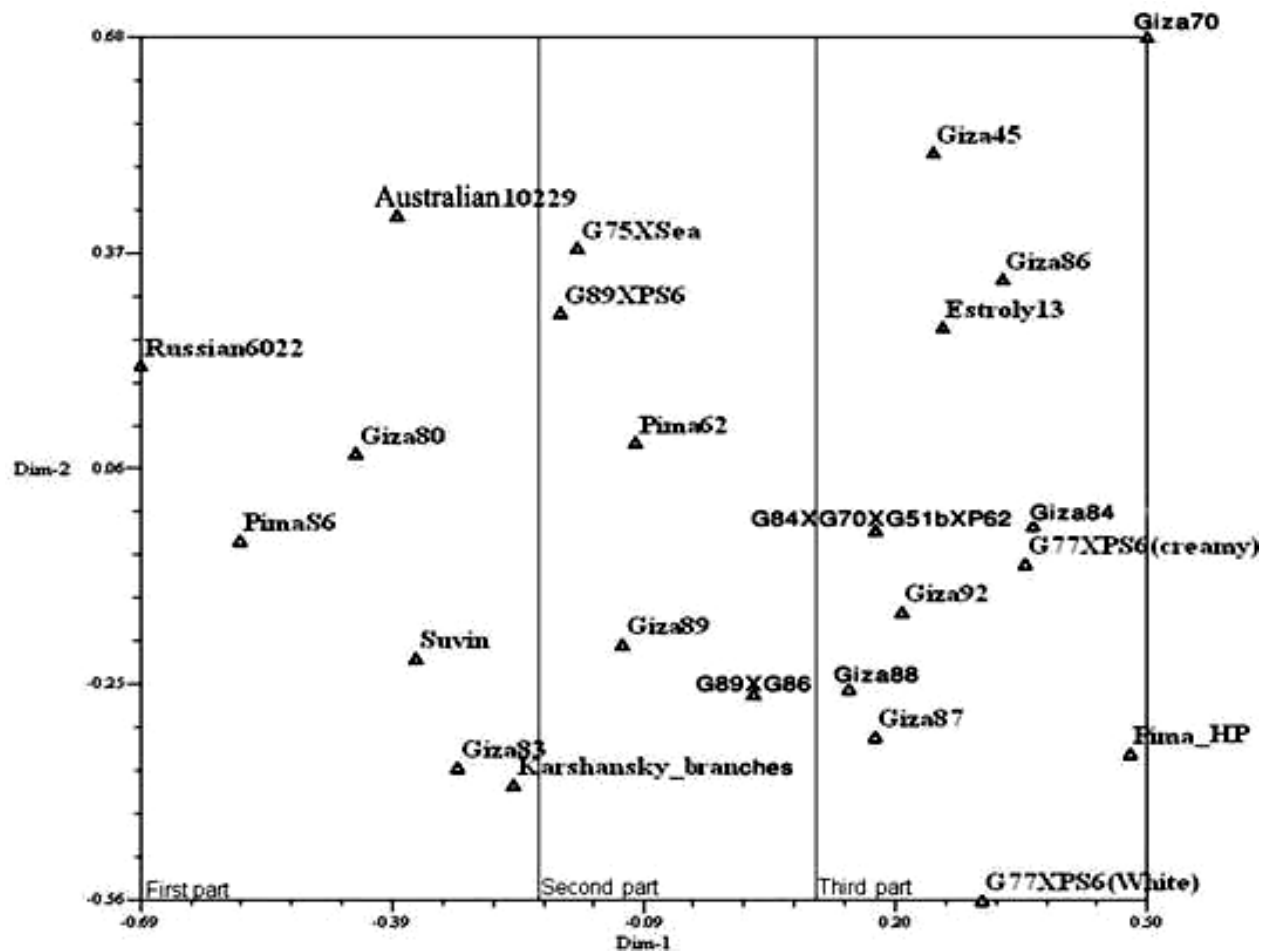


Figure 5. Two-dimensional plot of principal coordinate analysis of 24 cotton genotype based on molecular markers (PC1 = 11.8, PC2 = 10.6).

(especially EST-SSR markers) were more accurate in determining the genetic diversity than ISSR markers. The association between molecular and morphological markers was poor (data not shown) and may be referred to the habit of the morphological characteristics which was affected by the environmental conditions, so that the molecular results could be more reliable than the morphological traits.

REFERENCES

- Abd-El-Haleem SHM, Metwali EMR, Al-Felaly AMM (2010). Genetic Analysis of Yield and its Components of Some Egyptian Cotton (*Gossypium barbadense* L.) Varieties. *World J. Agric. Sci.* 6(5):615-621.
- Abdellatif KF, Khidr YA, El-Mansy YM, El-Lawendy MM, Soliman YA (2012). Molecular diversity of Egyptian cotton (*Gossypium barbadense* L.) and its relation to varietal development. *J. Crop Sci. Biotech.* 15: 93-99. DOI No. 10.1007/s12892-011-0120-5.
- Abdel-Salam ME (1999). *The Egyptian Cotton: Production, Quality and Marketing*. Elkalema Press, 4, Ahmed Barada St. Giza-Cairo, Egypt.
- Anderson JA, Sorrells ME, Tanksley SD (1993). RFLP analysis of genomic regions associated to preharvest sprouting in wheat. *Crop Sci.* 33:453-459.
- Cornelius BK, Sneller CH (2002). Yield and molecular diversity of soybean lines derived from crosses between Northern and Southern elite parents. *Crop Sci.* 20:187-190.
- Dellaporta SL, Wood J, Hicks JB (1983). A plant DNA miniprep preparation version II. *Plant Mol. Biol. Report.* 1:19-21.
- El-Mansy YM, El-Lawendy MM, Soliman YA, Khidr YA, Abdellatif KF (2012). Cotton germplasm diversity and its relation to varietal improvement as revealed by agronomic characters and SSR markers technique. *Minf. J. Agric. Res.* 37:337-349.
- Esmail RM (2007). Genetic analysis of yield and its contributing traits in two intra-specific cotton crosses. *J. Appl. Sci. Res.*, 3(12):2075-2080.
- Esmail RM, Zhang JF, Abdel-Hamid AM (2008). Genetic Diversity in Elite Cotton Germplasm Lines Using Field Performance and Rapid Markers. *World J. Agric. Sci.* 4(3): 369-375.
- Hamoued HME, Yehia WMB (2009). Utilization of selection index as tool to improve selection efficiency in cotton. *Egypt. J. Appl. Sci.* 24 (1): 226-239.
- Hendawy FA (1994). Quantitative inheritance of seed cotton yield and some of its components. *Menofiya J. Agric. Res.* 19(5): 2287-2300.
- Liu B, Wendel JF (2001). Intersimple sequence repeat (ISSR) polymorphisms as a genetic marker system in cotton. *Mol. Ecol. Notes* 1:205-208.
- Mahros EH (2008). Triple test cross analysis in some cotton crosses.

- M.Sc., Thesis, Menofiya Univ., Egypt.
- Milligan SB, Bbalzarini M, White WH (2003). Broad-sense heritability, genetic correlations, and selection indices for sugarcane borer resistance and their relation to yield loss. *Crop Sci.* 43:1729-1735.
- Mohammadi SA, Prasanna BM (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. *Crop Sci.* 43:1235-1248.
- Morgante M, Hanafey M, Powell W (2002). Microsatellites are preferentially associated with non-repetitive DNA in plant genomes. *Nat. Genet.* 30:194-200.
- Preetha S, Raveendren TS (2008). Molecular marker technology in cotton. *Biotechnol. Mol. Biol. Rev.* 3:032-045.
- Park YH, Alabady MS, Ulloa M, Sickler B, Wilkins TA, Yu J, Stelly DM, Kohel RJ, el-Shihy OM, Cantrell RG (2005). Genetic mapping of new cotton fiber loci using EST-derived microsatellites in an interspecific recombinant inbred line cotton population. *Mol. Genet. Genomics* 274:428-441.
- Rana MK, Bhat KV (2005). RAPD marker for genetic diversity study among Indian cotton cultivars. *Curr. Sci.* 88:1956-1961.
- Reddy UK, Pepper A, Abdurakhmonov IY, Saha S, Jenkins JN, Brooks T, Bolek Y, El-Zik K (2001). New dinucleotide and trinucleotide microsatellite marker resources for cotton genome research. *J. Cotton Sci.* 5:103-113.
- Rohlf FJ (1998). NTSYSpc. Numerical taxonomy and multivariate analysis system, version 2.02c. Exeter Software, New York.
- Singh P, Narayanan SS (2000). Biometrical techniques in plant breeding 2nd ed. Kalyani publishers, Ludhiana, New Delhi, Noid, India
- Sneath P, Sokal R (1973). Numerical Taxonomy, Freeman, San Francisco. 12:102-108.
- Sokal R, Sneath P (1963). Principles of Numerical Taxonomy, San Francisco: W.H. Freeman, 1963.
- Turkoglu Z, Bilgener S, Ercisli S, Bakir M, Koc A, Akbulut M, Gercekcioglu R, Gunes M, Esitken A (2010). Simple sequence repeat-based assessment of genetic relationships among Prunus rootstocks. *Genet. Mol. Res.* 9:2156-2165.
- Zietkiewicz E, Rafalski A, Labuda D (1994). Genomic fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics.* 20:176-183.
- Zhang HB, Li Y, Wang B, Chee PW (2008). Recent advances in cotton genomics. *Int. J. Plant Genomics* doi:10.115/2008/742304. 20 pp. Hindawai Publishing Corporation.
- Zhang YX, Lin ZX, Li W, Tu LL, Nie YC, Zhang XL (2007). Studies of new EST-SSRs derived from *Gossypium barbadense*. *Chin. Sci. Bull.* 52:2522-2531.
- Zhang Y, Wang XF, Li ZK, Zhang GY, Ma ZY (2011). Assessing genetic diversity of cotton cultivars using genomic and newly developed expressed sequence tag-derived microsatellite markers. *Genet Mol. Res.* 10(3):1462-70.