



# Inter simple sequence repeats and morphological traits to identify cultivated cotton varieties (*Gossypium barbadense* L.) in Egypt

Hoida Zaki · Nagwa R. A. Hussein

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**Abstract** Egyptian cultivated Cotton significantly impacted Egypt's economy, as it is well-known worldwide. This study aims to determine how much genetic and phenotypic variation exists in five different varieties of Egyptian Cotton using Inter Simple Sequence Repeats (ISSR) as a molecular marker and twenty-one quantitative and qualitative morphological traits as a taxonomic source in the development and evolution of this plant. Eleven ISSR primers were used, producing a total of 134 bands with a polymorphism percentage of 67%. Positive and negative significant Pearson correlations were found among the studied morphological traits in line with the phenotypic correlations in some characteristics. The genotypic correlation coefficient was higher in magnitude than that of phenotypic correlation. The five varieties were grouped into two major clusters using the UPGMA method based on morphological and ISSR analysis. The first one included G86 and G89 varieties, while the second cluster included G80 and G95; the G90 was separated from the other four varieties. This genetic relationship may be attributed to their similar ancestors. The information from this study should help with cotton breeding efforts to attain a

high level of germplasm diversity and develop new high-yielding types to enhance cotton production and quality.

**Keyword** Egyptian cotton · *Gossypium barbadense* · Genetic diversity · ISSR · Genotypic and phenotypic correlations

## Introduction

The genus *Gossypium* (Malvaceae family) exhibits great phenotypic variation and includes approximately 50 species (Campbell et al. 2009). Because of its high economic significance, the genus has gained much attention from taxonomists, evolutionary biologists, and agricultural scientists (Wendel et al. 2009). Some *Gossypium* (G.) species are known for morphological resemblance, making it difficult to identify them, especially if fruits are not present (Stanton et al. 1994). The widely cultivated Cotton belongs to the two species *Gossypium barbadense* and *Gossypium hirsutum* L. domesticated in South America. The new cultivars of the two species are introgressed to each other (Wendel et al. 2009). Cotton is the most widely used natural fiber in the world. However, the cultivars grown for commercial purposes are nearly the species *G. hirsutum*, derived from only a few cultivars and hence have a limited genetic base (Moiana et al. 2015).

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H. Zaki (✉) · N. R. A. Hussein  
Botany and Microbiology Department, Faculty of Science,  
South Valley University, Qena 83523, Egypt  
e-mail: hoaida.zaki@sci.svu.edu.eg

N. R. A. Hussein  
e-mail: nagwa\_hussein@sci.svu.edu.eg

On the other hand, *G. barbadense* is the second most cultivated species, known for the best fiber quality (Liu et al. 2015). Cotton is a fiber and oil crop with a good fiber texture and high-quality oil (Aslam et al. 2020). Cotton is a major source of income and employment in many nations, with millions of people employed in crop production, processing, and distribution (Chaudhry et al. 2003). Egyptian and Pima cotton are grown for extra-long, strong, and fine fiber (Hussein et al. 2007).

In Egypt, Cotton provides a source of income for millions of people who work in the textile sector, either directly or indirectly. Egyptian cotton breeders are concentrating their efforts on improving yield for long-staple Cotton and attempting to develop new, improved varieties with desirable traits for both farmers and breeders are continuous (Yehia and El-Hashash 2019). Breeders improved the upper half mean length, uniformity index, and strength of these Cotton, determined by the longest staple length in the long-staple category. Furthermore, the strength level is comparable to or near that of extra-long-staple Cotton allowing long-staple Cotton to compete in spinning performance and yarn quality with extra-long-staple Cotton (Abdelbary et al. 2021).

Plant morphological traits are continuously used to assess the genetic variability between and within crop plant populations (Begna 2021). The Genetic diversity of Cotton is important for sustainable development for breeding new genotypes, and it is also critical to select parents for plant breeding programs (Bertan et al. 2007). The first step in producing germplasm and crop cultivars is to characterize genetic diversity and the degree of connection between and within genetic resources, which are regarded as sources for new crop varieties (Govindaraj et al. 2015; Han et al. 2022) and essential for the crop improvement success (Rana et al. 2007). Genetic diversity data is vital when attempting to improve crops and develop new varieties (Bakhsh et al. 2019; Pereira et al. 2015; Swarup et al. 2021).

The use of molecular markers has become an important additional requirement for understanding the genetic basis in addition to sets of morphological traits (Selvi et al. 2013). Molecular markers have been used to measure the genetic diversity and relationships between species and their wild relatives in Cotton (Ditta et al. 2018; Hoffmann et al. 2018; Saif et al. 2017; Sethi et al. 2015; Tidke et al. 2014).

Polygenic morphological traits are influenced by the environment and are mostly quantitatively inherited (Hassan 2018; Lukonge et al. 2007). Inter Simple Sequence Repeat (ISSR) has been applied in many genetic diversity studies. ISSR is a simple and informative genetic marker system in Cotton for revealing inter- and intraspecific variation (Abdellatif et al. 2012; Farahani et al. 2018; Kahodariya et al. 2015; Liu and Wendel 2001). It uses the primers complementary to a single SSR and anchored at either the 5' or 3' end with a one- to three-base extension. The ISSR markers are robust, reliable, quick, efficient, and reproducible, with greater discriminative ability than the other techniques (Abdellatif et al. 2012; Dongre et al. 2007; Preetha and Raveendren 2008; Rana et al. 2007).

ISSR markers have been used for differentiating cotton genotypes. For example, the cotton genotypes (*G. barbadense* L.) were clustered into two major clusters using a UPGMA cluster analysis based on ISSR polymorphism, according to Hoffmann et al. (2018). Also, they concluded that the *G. barbadense* germplasm had a narrow genetic diversity, and the genetic relationship was attributed to their similar ancestors. The present study aims to analyze the genetic diversity among Cotton (*G. barbadense*) varieties using Inter-Simple Sequence Repeat markers (ISSR), evaluate the variation in morphological traits to differentiate five varieties of Egyptian Cotton and estimate the genetic distance between them.

## Material and methods

### Plant material

Five Egyptian Giza varieties of *Gossypium barbadense* L. were involved in this study. The varieties' names, abbreviations, pedigrees, and the year of release of these genotypes are illustrated in Table 1. Seeds were kindly provided by the Cotton Research Institute, El-Marashda Research Station, Agricultural Research Center-Egypt. Plants of the 2nd generation are grown under newly reclaimed lands in El-Marashda city, Qena governorate, Egypt. The second author prepares a herbarium collection. The specimens are dried and kept in Botany & Microbiology Department—South Valley University Herbarium (QNA- proposed acronym). The

**Table 1** Varieties names, Abbreviations, pedigrees, and the year of release of the studied cotton genotypes

| No | Genotype | Abbreviation | Pedigrees                | Year of release |
|----|----------|--------------|--------------------------|-----------------|
| 1  | Giza 80  | G 80         | Giza 66×Giza 73          | 1981            |
| 2  | Giza 86  | G 86         | Giza 75×Giza 81          | 1995            |
| 3  | Giza 89  | G 89         | Giza 75×6022             | 1997            |
| 4  | Giza 90  | G 90         | Dendera×Giza 83          | 2000            |
| 5  | Giza 95  | G 95         | [(Giza 83×Giza 75)]×5844 | 1999            |

\*Pedigrees and year of release are followed after (Abdellatif et al. 2012) and (El-Adly et al. 2018)

nomenclature and synonyms of *G. barbadense* L. are reviewed according to [www.tropicos.org](http://www.tropicos.org); after referring to (Fryxell 1969), a specimen sheet of it is revised from a collection of the National History Museum- London, Appendix 1.

### Morphological characters

This study concerns some vegetative and reproductive, quantitative, and qualitative morphological characters. They are also investigated based on the crop yield of these genotypes and previously prepared herbarium specimens. The quantitative characters of the stem and leaves are the stem height (cm), no. of vegetative branches, no. of fruiting branches, the position of the 1st fruiting branch in relation to the node order, petiole length, leaf length, leaf wide (cm), no. of leaf lobes, lobes length and lobe width (cm). The qualitative characteristics of the stem are its hairiness, color, black spots amount, outline shape, petiole color, leaf bract shape, flower color, internal petaloid spots' color, staminal tube length, stigma height in relation to anthers, and the anthers color. Three replicates of specimens for each genotype are examined—fifteen readings for each specimen record observations and reading of the characters.

### ISSR-PCR reactions

Eleven ISSR primers were used to detect polymorphism. The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (Sigma), 2.5 µl primer (10pcmol), 3 µl template DNA (10 ng), and 7 µl dH<sub>2</sub>O, according to (Adhikari et al. 2015).

### Thermocycling profile PCR

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (P.E. Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle was composed of a denaturation step lasting 1 min at 94 °C, an annealing step lasting 1 min at 45 °C, and an elongation step lasting 1.5 min at 72 °C. In the final cycle, the primer extension segment was extended to 7 min at 72°C.

### Detection of the PCR products

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5µg/ml) in a 1X TBE buffer at 95 V. PCR products were visualized on U.V. light and photographed using a Gel Documentation System (BIO-RAD 2000).

### Data analysis

The morphological measurements represent the means with standard error mean (SEM), and an Analysis of Variance (one-way ANOVA) of all the morphological measurements was performed using the XLSTAT software (Addinsoft 2021) following Steel and Torrie's (1997) method. The mean comparison of the treatments was investigated using the L.S.D test at the level of significance ( $p < 0.05$ ). Correlation coefficient (Pearson) values between morphological traits were obtained using the SPSS program version-20 (Dunn 2013). The environmental, phenotypic, and genotypic coefficients of variations and their variance were estimated according to Singh and Chaudhary (1985), and the heritability (broad sense) was determined based on the genetic mean

according to Allard (1999). The genetic Advance was calculated per the formula by Johnson et al. (1955) using the variability package of R statistical software in RStudio version 1.4.1717 (Popat 2020).

For ISSR analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples, and the final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. The PAST 3.22 software (Hammer et al. 2001) was used to construct cluster trees (dendrogram) according to the Euclidean distance coefficient using the unweighted pair group method with arithmetic averages (UPGMA).

The potential of the ISSR markers in the estimation of genetic variability was assessed by measuring the Heterozygosity index (H); Polymorphic Information Content (PIC); Effective multiplex ratio (E); Arithmetic mean of H (H.av); Marker Index (MI); Discriminating power (D); Resolving power (R) according to (Amiryousefi et al. 2018).

## Results

### Morphological characters

The detailed data in Table 2 show valuable variation in the morphological traits among the five varieties of *G. barbadense*. The plant stem was mostly green or greenish-red, rarely green to red herbs; stems outline circular, polygonal, or circular to polygonal, glabrous, with few to dense black spots on stems (dense in G 89). The stem height ranged from 82 cm to 103.2 cm in G 89 and G 95, respectively. Giza 90 genotype showed the lowest measurements in most quantitative traits, including No. of branches bearing fruits ( $16.33 \pm 0.56$ ), the position of 1st branch bearing fruit in relation to nodes ( $6.60 \pm 0.51$ ), Petiole L ( $6.15 \pm 0.15$  cm), Leaf width ( $6.49 \pm 0.24$  cm), and Lobe length ( $3.77 \pm 0.40$  cm). Giza 86 genotype exhibited the highest values in some morphological characters such as No. of branches bearing fruits ( $19.53 \pm 0.67$ ), the position of 1st branch bearing fruit in relation to nodes ( $9.07 \pm 0.28$ ), and Petiole L ( $7.61 \pm 0.24$ ), while the Giza 80 genotype showed the highest values in the Leaf length ( $7.69 \pm 0.25$ ), lobe length ( $4.58 \pm 0.25$ ), and lobe width ( $3.15 \pm 0.17$ ). Leaves were bracteate, bract

linear-lanceolate; petiolate, green, greenish-red or green to greenish-red; flowers were yellow; petals' basal internal spots deep purple in G 86 and G 89 varieties and ranges between length or deep purple in other varieties; staminal tube in all genotypes is short; anthers color shades are orange/yellowish orange or purplish yellow, brilliant yellow tending to orange or orange to purplish orange and yellowish-orange to brilliant or purplish yellow; stigma commonly higher than the anthers' height.

Key for *G. barbadense* L. varieties based on morphological characters

|     |                                                                   |      |
|-----|-------------------------------------------------------------------|------|
| 1.a | Stem outline polygonal, with few black spots...                   | G 80 |
| b   | Stem outline not polygonal, with rare/few to dense black spots... | 2    |
| 2.a | The average no. of vegetative branches is 1.4–1.5...              | 3    |
| b   | The average no. of vegetative branches is > 2.0...                | G 90 |
| 3.a | Lobe/leaf L. ratio = 0.5...                                       | 4    |
| b   | Lobe/leaf L. ratio more than 0.5...                               | G 89 |
| 4.a | Anthers orange or purplish yellow...                              | G 95 |
| b   | Anthers yellowish orange or purplish orange...                    | G 86 |

Table 3 shows the Pearson correlation coefficient between each pair of morphological traits based on cotton varieties. As shown in Table 3, there was a highly positive significant correlation between leaf length and leaf width ( $0.529^{**}$ ), between stem height and No. of branches bearing fruits ( $0.477^{**}$ ), and between leaf width and lobe width ( $0.443^{**}$ ). In the meantime, the position of the 1st branch bearing fruit in relation to nodes had a significant positive correlation with each of No. of branches bearing fruits ( $0.278^{*}$ ), Leaf width ( $0.250^{*}$ ), and Lobe width ( $0.293^{*}$ ). Also, the petiole L significantly and positively correlated with Leaf length ( $0.280^{*}$ ) and Leaf width ( $0.255^{*}$ ). Other negative significant correlations were found between No. of vegetative branches, and each of No. of branches bearing fruits ( $-0.261^{*}$ ) and Petiole L ( $-0.246^{*}$ ) and between Stem height and Lobe length ( $-0.238^{*}$ ). Also, a significant

**Table 2** Some vegetative and reproductive morphological characters of five varieties of *G. barbadense* L

| No | Character                                                 | Genotypes                                        |                                     |                                    |                                       |                                                | LSD 5%              |
|----|-----------------------------------------------------------|--------------------------------------------------|-------------------------------------|------------------------------------|---------------------------------------|------------------------------------------------|---------------------|
|    |                                                           | G80                                              | G86                                 | G89                                | G90                                   | G95                                            |                     |
| 1  | Stem height (cm)                                          | 89.67±2.99                                       | 96±3.59                             | 82±2.23                            | 89.33±3.68                            | 103.2±3.02                                     | 8.867               |
| 2  | No. of vegetative branches                                | 1.40±0.34                                        | 1.53±0.31                           | 1.47±0.26                          | 2.07±0.54                             | 1.53±0.29                                      | 1.014               |
| 3  | No. of branches bearing fruits                            | 16.67±0.82                                       | 19.53±0.67                          | 16.33±0.97                         | 16.33±0.56                            | 19.27±0.50                                     | 2.043               |
| 4  | Position of 1st branch bearing fruit in relation to nodes | 7.73±0.23                                        | 9.07±0.28                           | 7.20±0.33                          | 6.60±0.51                             | 8.07±0.45                                      | 1.055               |
| 5  | Petiole L (cm)                                            | 7.21±0.43                                        | 7.61±0.24                           | 6.25±0.19                          | 6.15±0.15                             | 6.82±0.35                                      | 0.823               |
| 6  | Leaf length (cm)                                          | 7.69±0.25                                        | 6.60±0.12                           | 7.34±0.13                          | 7.33±0.19                             | 7.20±0.07                                      | 0.46                |
| 7  | Leaf width (cm)                                           | 6.93±0.21                                        | 6.83±0.23                           | 6.60±0.20                          | 6.49±0.24                             | 7.00±0.17                                      | 0.592               |
| 8  | No. of leaf lobes                                         | 3.40±0.16                                        | 3.27±0.12                           | 3.43±0.17                          | 3.33±0.13                             | 3.20±0.11                                      | 0.389               |
| 9  | Lobe length (cm)                                          | 4.58±0.35                                        | 3.89±0.36                           | 4.48±0.21                          | 3.77±0.40                             | 3.82±0.20                                      | 0.887               |
| 10 | Lobe width (cm)                                           | 3.15±0.17                                        | 3.09±0.14                           | 2.88±0.13                          | 2.92±0.10                             | 3.11±0.08                                      | 0.365               |
| 11 | Stem hairiness                                            | Glabrous                                         | Glabrous                            | Glabrous                           | Glabrous                              | Glabrous                                       | QUALITA-TIVE traits |
| 12 | Stem color                                                | Green or greenish red                            | Green or greenish-red or to red     | Green or greenish red              | Green or greenish-red                 | Green or greenish-red                          |                     |
| 13 | Stem black spots                                          | Few                                              | Few or few to dense                 | Dense or few                       | Rare to few                           | Few to dense                                   |                     |
| 14 | Stem outline shape                                        | Polygonal                                        | Circular or circular to polygonal   | Polygonal or circular to polygonal | Polygonal or circular to polygonal    | Circular or polygonal or circular to polygonal |                     |
| 15 | Petiole color                                             | Green or greenish red                            | Green or green to greenish red      | Greenish-red or tend to green      | Greenish-red or green to Greenish-red | Green to greenish-red                          |                     |
| 16 | Bract shape                                               | Linear-lanceolate                                | Linear-lanceolate                   | Linear-lanceolate                  | Linear-lanceolate                     | Linear-lanceolate                              |                     |
| 17 | Flower color                                              | Yellow                                           | Yellow                              | Yellow                             | Yellow                                | Yellow                                         |                     |
| 18 | Internal petaloid spots color                             | Light or Deep purple                             | Deep purple                         | Deep purple                        | Deep or light purple                  | Deep or light purple                           |                     |
| 19 | Staminal tube length                                      | Short                                            | Short                               | Short                              | Short                                 | Short                                          |                     |
| 20 | Stigma height in relation to anthers                      | Higher                                           | Higher                              | Higher                             | Higher                                | Higher                                         |                     |
| 21 | Anther's color                                            | Yellowish orange to brilliant or purplish yellow | Yellowish orange or purplish yellow | Brilliant yellow or tend to orange | Orange or purplish-orange             | Orange or purplish-yellow                      |                     |

negative correlation was found between No. of leaf lobes and the Lobe width ( $-0.264^*$ ).

Sum Square, Mean Square, F-value, and probability value from Analysis of Variance for investigated traits in the examined *G. barbadense* varieties are

presented in Table 4. The results revealed that Stem height, the position of 1st branch bearing fruit in relation to nodes, and Leaf length were highly significant differences as probability value  $Pr(>F)$  was  $\leq 0.001$ . Meanwhile, No. of branches bearing fruits and Petiole

**Table 3** Pearson correlation coefficient between each pair of morphological traits based on the examined *G. barbadense* varieties

| Character                                                 | Stem height | No. of vegetative branches | No. of branches bearing fruits | Position of 1st branch bearing fruit in relation to nodes | Petiole L | Leaf length | Leaf width | No. of leaf lobes | Lobe length | Lobe width |
|-----------------------------------------------------------|-------------|----------------------------|--------------------------------|-----------------------------------------------------------|-----------|-------------|------------|-------------------|-------------|------------|
| Stem height                                               | 1           | − 0.068                    | 0.477                          | 0.062                                                     | 0.219     | 0.023       | 0.018      | − 0.159           | − 0.238*    | − 0.062    |
| No. of vegetative branches                                | − 0.068     | 1                          | − 0.261*                       | − 0.204                                                   | − 0.246*  | − 0.141     | 0.055      | − 0.149           | 0.163       | 0.074      |
| No. of branches bearing fruits                            | 0.477**     | − 0.261*                   | 1                              | 0.278*                                                    | 0.211     | 0.024       | 0.103      | 0.011             | − 0.034     | − 0.116    |
| Position of 1st branch bearing fruit in relation to nodes | 0.062       | − 0.204                    | 0.278*                         | 1                                                         | 0.22      | 0           | 0.25*      | 0.057             | − 0.063     | 0.293*     |
| Petiole L                                                 | 0.219       | − 0.246*                   | 0.211                          | 0.22                                                      | 1         | 0.28*       | 0.255      | 0.042             | − 0.172     | 0.074      |
| Leaf length                                               | 0.023       | − 0.141                    | 0.024                          | 0                                                         | 0.28*     | 1           | 0.529**    | 0.092             | − 0.029     | 0.223      |
| Leaf width                                                | 0.018       | 0.055                      | 0.103                          | 0.25*                                                     | 0.255*    | 0.529**     | 1          | − 0.101           | 0.109       | 0.443**    |
| No. of leaf lobes                                         | − 0.159     | − 0.149                    | 0.011                          | 0.057                                                     | 0.042     | 0.092       | − 0.101    | 1                 | 0.183       | − 0.264*   |
| Lobe length                                               | − 0.238*    | 0.163                      | − 0.034                        | − 0.063                                                   | − 0.172   | − 0.029     | 0.109      | 0.183             | 1           | 0.206      |
| Lobe width                                                | − 0.062     | 0.074                      | − 0.116                        | 0.293*                                                    | 0.074     | 0.223       | 0.443**    | − 0.264*          | 0.206       | 1          |

\*\*Correlation is significant at the 0.01 level (2-tailed)

\*Correlation is significant at the 0.05 level (2-tailed)

**Table 4** Sum Square, Mean Square, F-value, and probability value from Analysis of Variance in the examined *G. barbadense*

Signif. codes: \*Significant at 0.05 level, \*\*Significant at 0.001 level, \*\*\*Significant at 0.0001 level

Degree of freedom (df) = 4

| Characters                                                | Sum Sq | Mean Sq | F value | Pr (> F)    |
|-----------------------------------------------------------|--------|---------|---------|-------------|
| Stem height                                               | 3809.8 | 952.45  | 6.7436  | 0.000167*** |
| No. of vegetative branches                                | 4.267  | 1.0667  | 0.5443  | 0.7038      |
| No. of branches bearing fruits                            | 158.88 | 39.72   | 4.9837  | 0.001654**  |
| Position of 1st branch bearing fruit in relation to nodes | 51.867 | 12.9667 | 5.9846  | 0.000441*** |
| Petiole L                                                 | 23.21  | 5.8025  | 4.657   | 0.002573**  |
| Leaf length                                               | 9.4168 | 2.3542  | 5.5912  | 0.000737*** |
| Leaf width                                                | 2.847  | 0.7118  | 1.1417  | 0.3464      |
| No. of leaf lobes                                         | 0.6667 | 0.16667 | 0.7216  | 0.58079     |
| Lobe length                                               | 9.077  | 2.26913 | 1.3661  | 0.2573      |
| Lobe width                                                | 0.8755 | 0.21887 | 0.9341  | 0.4509      |

L were highly significant differences among varieties ( $P$  value  $\leq 0.01$ ), while the other traits showed non-significant differences among the studied varieties.

The data for the Analysis of Variance in the examined *G. barbadense* varieties are shown in Table 5. The data showed that the environmental variance, genotypic variance, phenotypic variance, heritability (in the broad sense), and genetic advance values for the stem height trait were all the highest. The number of vegetative branches was the trait with the highest values for the environmental, genotypic, and phenotypic

coefficient of variation. As for heritability, the data indicated that heritability was the highest value for stem height, followed by the position of 1st branch bearing fruit in relation to nodes, leaf length, the number of branches bearing fruits, and Petiole length.

#### Phenotypic and genotypic correlations

Phenotypic and Genotypic Correlation in the quantitative characters of the examined *G. barbadense* genotypes were calculated among all characters (Table 6)

**Table 5** Estimates of variance components and genetic parameters for various quantitative traits in the examined *G. barbadense* varieties

| No | Character                                                 | Environmental Variance | Genotypic Variance | Phenotypic Variance | Environmental Coefficient of Variance | Genotypic Coefficient of Variance | Phenotypic Coefficient of Variance | Heritability (Broad Sense) | Genetic Advance | Genetic Advance as a percentage of mean |
|----|-----------------------------------------------------------|------------------------|--------------------|---------------------|---------------------------------------|-----------------------------------|------------------------------------|----------------------------|-----------------|-----------------------------------------|
| 1  | Stem height (cm)                                          | 141.23                 | 54.08              | 195.32              | 12.91                                 | 7.99                              | 15.18                              | 0.277                      | 7.97            | 8.66                                    |
| 2  | No. of vegetative branches                                | 1.95                   | -0.059             | 1.9                 | 87.48                                 | 0                                 | 86.15                              | 0                          | 0               | 0                                       |
| 3  | No. of branches bearing fruits                            | 7.97                   | 2.11               | 10.08               | 16.02                                 | 8.25                              | 18.02                              | 0.21                       | 1.37            | 7.78                                    |
| 4  | Position of 1st branch bearing fruit in relation to nodes | 2.16                   | 0.72               | 2.88                | 19.03                                 | 10.97                             | 21.97                              | 0.25                       | 0.87            | 11.29                                   |
| 5  | Petiole L (cm)                                            | 1.24                   | 0.303              | 1.55                | 16.39                                 | 8.09                              | 18.28                              | 0.19                       | 0.503           | 7.38                                    |
| 6  | Leaf length (cm)                                          | 0.42                   | 0.129              | 0.55                | 8.97                                  | 4.96                              | 10.25                              | 0.23                       | 0.35            | 4.95                                    |
| 7  | Leaf width (cm)                                           | 0.62                   | 0.01               | 0.63                | 11.66                                 | 1.13                              | 11.72                              | 0.01                       | 0.02            | 0.23                                    |
| 8  | No. of leaf lobes                                         | 0.23                   | -0.01              | 0.23                | 14.41                                 | 0                                 | 14.28                              | 0                          | 0               | 0                                       |
| 9  | Lobe length (cm)                                          | 1.66                   | 0.04               | 1.70                | 31.37                                 | 4.89                              | 31.75                              | 0.02                       | 0.1             | 1.56                                    |
| 10 | Lobe width (cm)                                           | 0.23                   | -0.001             | 0.23                | 15.97                                 | 0                                 | 15.94                              | 0                          | 0               | 0                                       |

**Table 6** Phenotypic (above diagonal) and Genotypic (below diagonal) Correlations of the quantitative characters for the examined *G. barbadense*

| No | Character                                                 | Stem height (cm)             | No. of branches bearing fruits | Position of 1st branch bearing fruit in relation to nodes | Petiole L (cm)               | Leaf length (cm)             | Leaf width (cm)              | Lobe length (cm)             |
|----|-----------------------------------------------------------|------------------------------|--------------------------------|-----------------------------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| 1  | Stem height (cm)                                          | 1                            | <b>0.5137</b> **               | <b>0.0745</b> <sup>NS</sup>                               | <b>0.1497</b> <sup>NS</sup>  | <b>−0.1901</b> <sup>NS</sup> | <b>−0.1901</b> <sup>NS</sup> | <b>−0.1901</b> <sup>NS</sup> |
| 2  | No. of branches bearing fruits                            | 0.9648**                     | 1                              | <b>0.2848</b> *                                           | <b>0.1813</b> <sup>NS</sup>  | <b>−0.1022</b> <sup>NS</sup> | <b>0.148</b> <sup>NS</sup>   | <b>−0.0419</b> <sup>NS</sup> |
| 3  | Position of 1st branch bearing fruit in relation to nodes | 0.7276 <sup>NS</sup>         | 1.05*                          | 1                                                         | <b>0.2054</b> <sup>NS</sup>  | <b>−0.1603</b> <sup>NS</sup> | <b>0.1692</b> <sup>NS</sup>  | <b>0.0017</b> <sup>NS</sup>  |
| 4  | Petiole L (cm)                                            | 0.5947 <sup>NS</sup>         | 0.846 <sup>NS</sup>            | 1.1446*                                                   | 1                            | <b>0.2435</b> *              | <b>0.2767</b> *              | <b>−0.098</b> <sup>NS</sup>  |
| 5  | Leaf length (cm)                                          | <b>−0.4823</b> <sup>NS</sup> | <b>−0.97</b> **                | <b>−0.848</b> <sup>NS</sup>                               | <b>−0.6814</b> <sup>NS</sup> | 1                            | <b>0.6005</b> **             | <b>0.079</b> <sup>NS</sup>   |
| 6  | Leaf width (cm)                                           | 2.4007 <sup>NS</sup>         | 1.97 <sup>NS</sup>             | 2.0698 <sup>NS</sup>                                      | 2.0284 <sup>NS</sup>         | <b>−1.0826</b> *             | 1                            | <b>0.1913</b> <sup>NS</sup>  |
| 7  | Lobe length (cm)                                          | <b>−1.2688</b> <sup>NS</sup> | <b>−1.23</b> <sup>NS</sup>     | <b>−0.3311</b> <sup>NS</sup>                              | 0.1654 <sup>NS</sup>         | <b>−0.4823</b> <sup>NS</sup> | 2.4007 <sup>NS</sup>         | 1                            |

The sig. of phenotypic correlation was tested using t-test (two-tail). The degree of freedom used is (genotypes\*replication)—2

\*, \*\* Significant at 5 and 1% probability level, respectively

after excluding the traits with negative genotypic variance in Table 5. The data revealed that the phenotypic correlation ranged from 0.0017 to 0.5137, while the genotypic correlation ranged from 0.5947 to 2.4007. It was clear that the genotypic correlation was higher in magnitude than the phenotypic correlation. It is noteworthy that stem height has a highly significant positive correlation with the number of branches bearing fruits at phenotypic and genotypic levels. Also, the number of branches bearing fruits had a significant positive correlation with the position of 1st branch bearing fruit in relation to nodes at genotypic and phenotypic levels. The leaf length and width had a highly significant positive correlation only at a phenotypic level which corresponds to the Pearson correlation in Table 3. There was a leaf length significantly negative correlation with the number of branches bearing fruits ( $p < 0.01$ ) and Leaf width ( $p < 0.05$ ) only at genotypic correlation. The petiole length trait had a significant positive correlation with the position of 1st branch bearing fruit in relation to nodes at

the genotypic level and with the leaf length and Leaf width traits at the phenotypic level.

#### ISSR molecular marker analysis

As shown in Table 7 and Fig. 1, the 11 used primers produced 134 bands for the five cotton genotypes; 42 bands were polymorphic, 85 monomorphic, and 7 bands were unique. The primer ISSR-06 produced the highest number of bands (18), and primer ISSR-03 and ISSR-11 had the lowest number (9). The number of polymorphic bands varied between primers; the bands produced by the primers ISSR-06 showed the highest number of polymorphic bands (12 bands), while primers ISSR-01, ISSR-08, and ISSR-11 showed one polymorphic band (the lowest number of bands). The number of polymorphic bands and percentage of polymorphism in the ISSR profile of the 11 primers are given in Table 2. The percentages of polymorphism varied and reached 67% in ISSR-06.

Table 8 illustrates the marker parameters, Heterozygosity index (H); Polymorphic Information

**Table 7** Primers codes, number of polymorphic, monomorphic, unique bands, and polymorphism percentage calculated by the analysis of ISSR fingerprinting in the examined *G. barbadense* varieties

| Primer  | Sequence                   | Monomorphic bands | Polymorphic (without Unique) | Unique bands | Polymorphic (with Unique) | Total number of bands | Polymorphism (%) | Mean of band frequency |
|---------|----------------------------|-------------------|------------------------------|--------------|---------------------------|-----------------------|------------------|------------------------|
| ISSR-01 | 5'-AGAGAGAGAGAGAGA GYC-3'  | 11                | 1                            | 0            | 1                         | 12                    | 8                | 0.98                   |
| ISSR-02 | 5'-AGAGAGAGAGAGAGA GYG-3'  | 9                 | 4                            | 2            | 6                         | 15                    | 40               | 0.81                   |
| ISSR-03 | 5'-ACACACACACACACA CYT-3'  | 6                 | 3                            | 0            | 3                         | 9                     | 33               | 0.82                   |
| ISSR-04 | 5'-ACACACACACACACA CYG-3'  | 9                 | 4                            | 1            | 5                         | 14                    | 36               | 0.81                   |
| ISSR-05 | 5'-GTGTGTGTGTGTGTG TYG-3'  | 5                 | 5                            | 2            | 7                         | 12                    | 58               | 0.75                   |
| ISSR-06 | 5'-CGCGATAGATAGATA GAT-3'  | 6                 | 12                           | 0            | 12                        | 18                    | 67               | 0.76                   |
| ISSR-07 | 5'-GACGATAGATAGATA GATA-3' | 5                 | 5                            | 1            | 6                         | 11                    | 55               | 0.71                   |
| ISSR-08 | 5'-AGACAGACAGACAGA CGC-3'  | 8                 | 1                            | 1            | 2                         | 10                    | 20               | 0.90                   |
| ISSR-10 | 5'-GACAGACAGACAGAC AAT-3'  | 9                 | 3                            | 0            | 3                         | 12                    | 25               | 0.87                   |
| ISSR-11 | 5'-ACACACACACAC ACACA-3'   | 8                 | 1                            | 0            | 1                         | 9                     | 11               | 0.93                   |
| ISSR-12 | 5'-ACACACACACAC ACACC-3'   | 9                 | 3                            | 0            | 3                         | 12                    | 25               | 0.88                   |
| Total   |                            | 85                | 42                           | 7            | 49                        | 134                   |                  |                        |

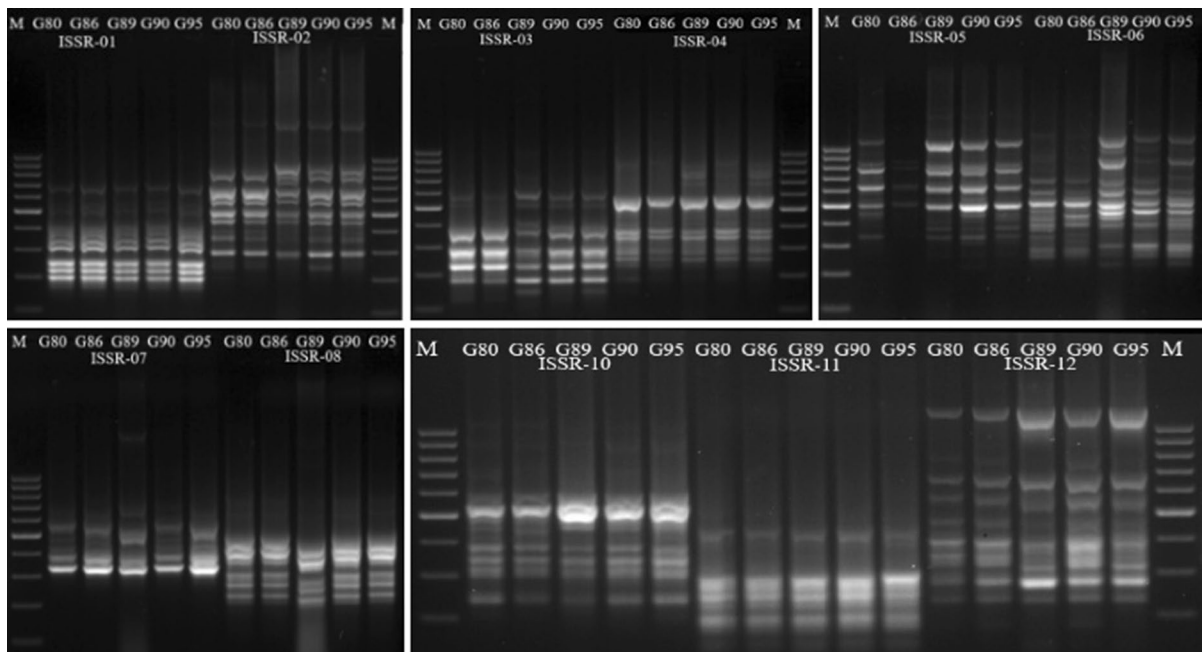
**Fig. 1** ISSR profile produced by 11 primers in the examined *G. barbadense* varieties

**Table 8** Marker parameters calculated for each ISSR primer used with the *G. barbadense* varieties

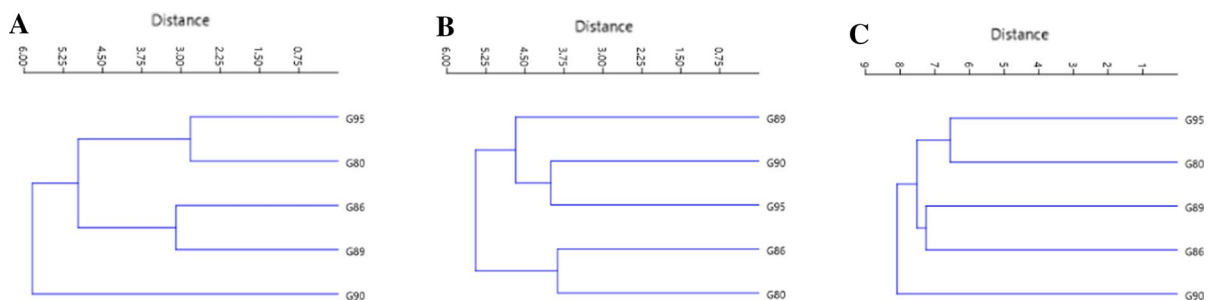
|             | H     | PIC   | E     | H.av   | MI     | D      | R    |
|-------------|-------|-------|-------|--------|--------|--------|------|
| ISSR-01     | 0.033 | 0.278 | 11.8  | 0.0005 | 0.0064 | 0.0333 | 0.4  |
| ISSR-02     | 0.304 | 0.232 | 12.2  | 0.0040 | 0.0494 | 0.3405 | 3.2  |
| ISSR-03     | 0.292 | 0.236 | 7.4   | 0.0065 | 0.0481 | 0.3273 | 2.4  |
| ISSR-04     | 0.302 | 0.233 | 11.4  | 0.0043 | 0.0493 | 0.3391 | 3.2  |
| ISSR-05     | 0.375 | 0.208 | 9     | 0.0063 | 0.0563 | 0.4407 | 3.6  |
| ISSR-06     | 0.369 | 0.210 | 13.6  | 0.0041 | 0.0558 | 0.4312 | 8    |
| ISSR-07     | 0.413 | 0.193 | 7.8   | 0.0075 | 0.0585 | 0.5010 | 4    |
| ISSR-08     | 0.180 | 0.262 | 9     | 0.0036 | 0.0324 | 0.1918 | 0.8  |
| ISSR-10     | 0.231 | 0.252 | 10.4  | 0.0039 | 0.0401 | 0.2508 | 2.4  |
| ISSR-11     | 0.124 | 0.271 | 8.4   | 0.0028 | 0.0232 | 0.1303 | 0.8  |
| ISSR-12     | 0.206 | 0.257 | 10.6  | 0.0034 | 0.0364 | 0.2215 | 2.4  |
| Avg./primer | 0.259 | 0.239 | 18.60 | 0.004  | 0.042  | 0.293  | 5.20 |

*H* Heterozygosity index, *PIC* Polymorphic Information Content, *E* Effective multiplex ratio, *H.av* Arithmetic mean of *H*, *MI* Marker Index, *D* Discriminating power, *R* Resolving power

Content (PIC); Effective multiplex ratio (E); Arithmetic mean of *H* (*H.av*); Marker Index (MI); Discriminating power (D), and Resolving power (R). The mean of PIC values is analyzed for all loci of each primer. A high PIC value of 0.278 (ISSR-01) and a low PIC value of 0.193 (ISSR-07) were obtained with an average PIC per primer of 0.239. The effective multiplex ratio (E) depends on the fraction of polymorphic fragments. Our study observed the highest effective multiplex ratio, 13.6, with the primer ISSR-06. The lowest effective multiplex ratio (E), 7.4, was observed with the primer ISSR-03, with an average E of 18.6 per primer. The MI (marker index) for each ISSR primer was calculated to determine the usefulness of the system of markers used. The highest MI was observed with three primers, ISSR-07, ISSR-05, and ISSR-06 (0.0585, 0.0563, and 0.0558, respectively), and the lowest in the primer ISSR-01 (0.0064), with an average MI of 0.042 per primer was obtained. The resolving power (R) parameter

indicates the discriminatory potential of the primers chosen. With an average R of 5.2 per primer, the primers with the greatest and lowest R-values were ISSR-06 and ISSR-01 (8 and 0.40, respectively). The expected heterozygosity *H<sub>E</sub>* of the diversity index *DI* is another name for the heterozygosity index (*H*). It is defined as the probability that an individual is heterozygous for the locus in the population. The highest value of the *H* index was observed with three primers, ISSR-07, ISSR-05, and ISSR-06 (0.413, 0.375, and 0.369, respectively), and the lowest in the ISSR-01 (0.033), with an average *H* of 0.259 per primer. The highest value of Discriminating power (D) was observed with the ISSR-07 primer (0.501) and the lowest with the primer ISSR-01 (0.033), with an average D of 0.293 per primer.

The UPGMA cluster analysis was constructed to illustrate the genetic behavior among the examined *G. barbadense* varieties based on morphological traits, ISSR polymorphism, and the combination of them

**Fig. 2** UPGMA cluster analysis of five *G. barbadense* varieties based on morphological traits (A), ISSR fingerprinting (B), and a combination of morphological & ISSR fingerprinting (C)

(Fig. 2A–C, respectively). Based on morphological traits and the combination between morphological traits and ISSR fingerprinting, the G90 was separated from the other four varieties that were grouped into two clusters; one cluster comprising G86 and G89 and the other cluster containing G80 and G95. The UPGMA tree illustrating the genetic diversity based on ISSR polymorphism separated the five varieties into two major clusters; the first one included three varieties, G90, G95, and G89, while the second cluster included G80 and G86.

## Discussion

The morphological description of Egyptian cotton genotypes indicated narrow differences in the qualitative traits. This is consistent with the results of many authors who used the morphological variation in cotton traits to divers among different genotypes of the plant, indicating their genetic diversity (Amer et al. 2016; El-Seidy et al. 2017; Hassan 2018; Hoffmann et al. 2018; Khan and Hassan 2011; Sen et al. 2022). (Hoffmann et al. 2018) found a significant variability for morphological characters in *Gossypium* accesses, underlining the possibility of using it to supplement breeding programs. Many positive and negative significant Pearson correlations were found between cotton genotypes in the study of Yehia and El-Hashash (2021). Many morphological characteristics of Cotton exhibited Pearson correlations between them, especially those about yield; this correlation positively or negatively was linked to allele accumulation and the plant's genetic potential (Hinze et al. 2011). The high Sum Square and Mean Square for some traits and the significance of the  $p$  value indicate the existence of considerable genetic divergence among the studied genotypes (Abd El-Moghny et al. 2015). This information could benefit cotton breeders looking to achieve a certain level of diversity in specific morphological traits. It could aid in the identification and engineering of crosses. Our results revealed that the heritability values were high for some quantitative traits, which is consistent with (Khan and Hassan 2011), which found high heritability values between different cotton genotypes and concluded that the desirable genotypes could be maintained

through simple selection in segregating generation. These results are in agreement with many studies for other plants found that a lesser magnitude of phenotypic correlation coefficients than the genotypic correlation coefficients (Baye et al. 2020; Dabi et al. 2016; Oladosu et al. 2018; Sen et al. 2022); this indicated that many morphological traits have underlying genetic relationships, and the environment less influences the phenotypic expression of these traits.

Analysis of diversity using PCR-based markers is an efficient and speedy approach to identifying genotype relationships and/or differences among genotypes (Rana et al. 2005; Schulman 2007; Zhao et al. 2015). In many studies on *Gossypium* genotypes and other plants, when compared to the different molecular markers utilized, ISSR markers produced the largest percentage of polymorphic bands (Abdellatif et al. 2012; Jedrzejczyk and Rewers 2020; Liu et al. 2006); it's believed that this is because ISSR is a dominant marker that measures the distance between two microsatellites. Variable factors like primer structure, template quantity, and the genome's lower proportion of annealing sites influence the number of bands amplified by different primers (Muralidharan and Wakeland 1993). In a similar study on the *G. barbadense* genotypes, (Saif et al. 2017) found that the lowest number of polymorphic fragments and percentage of polymorphism were reported for ISSR markers; in comparison, in our study, the percentage of polymorphism was given high values (ISSR's effectiveness). The PIC values were smaller than those obtained by (Abdellatif et al. 2012), whereas the PIC values of their study primers ranged from 0.75 to 0.94. The UPGMA of ISSR and morphological data combination indicated that varieties with the same ancestors were grouped together.

In conclusion, the cultivars employed in this study can be used as parents to expand the genetic basis of cotton germplasm in Egypt and for the development of new high-yielding types to enhance cotton production and quality.

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**Author contributions** HZ and NRAH conducted the research. NRAH performed the morphological data. ISSR data and analysis were performed by HZ. The two authors contributed to the final writing of the manuscript.

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## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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