

Study of genetic variation among some *Gossypium* spp. genotypes available in Iran using ISSR molecular marker

Received: 13.07.2020 / Accepted: 25.11.2020

Zahra Tajik Khaveh: PhD Student, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Alireza Iranbakhsh✉: Prof., Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran (iranbakhsh@iau.ac.ir)

Mosareza Vafaie Tabar: Research Associate Prof., Cotton Research Department, Tehran Agricultural and Natural Resources Research Center, Agricultural Research, Education and Extension Organization (AREEO), Varamin, Iran

Mostafa Ebadi: Assistant Prof., Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran

Abstract

The current study was conducted to explore genetic variations among several cotton species, including *Gossypium hirsutum* cultivar Varamin, *G. barbadense* cultivar T14, *G. herbaceum* cultivar Arya, hybrid of *G. hirsutum* cultivar Varamin × *G. barbadense* cultivar T14 (V×T14), and a hybrid of *G. hirsutum* cultivar Varamin × *G. herbaceum* cultivar Arya (V×A) using inter-simple sequence repeats (ISSR). In this study, a total of 60 bands were amplified using four ISSR marker primers. As a result, 57 bands showed polymorphism. The results showed a high level of diversity in the studied varieties. The highest percentage of polymorphism was related to ISSR4 primer with ACACACACACACT sequence (100%) and the lowest percentage was related to ISSR1 primer with AGAGAGAGAGAGAGC sequence (92.8%). Cluster analysis showed three major groups. The first group consisted of Arya and Varamin, the second group was comprised of V×A, T14 which showed a high genetic similarity indicating T14 originates from Varamin cultivar, and the third group contained V×T14. Overall, the results showed that, ISSR markers can be effectively used to study the genetic diversity of cotton.

Keywords: Cotton variations, cultivar, diploid, genetic diversity, *Malvaceae*, tetraploid

* بررسی تنوع ژنتیکی برخی از ژنوتیپ‌های موجود گونه‌های پنبه در ایران با استفاده از نشانگر مولکولی ISSR

دریافت: ۱۳۹۹/۰۴/۲۳ / پذیرش: ۱۳۹۹/۰۹/۰۵

زهرا تاجیک خاوه: دانشجوی دکتری، گروه زیست‌شناسی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران
علیرضا ایرانبخش✉: استاد پژوهش، گروه زیست‌شناسی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران (iranbakhsh@iau.ac.ir)
موسی الرضا وفایی تبار: دانشیار پژوهش، بخش تحقیقات پنبه و گیاهان لیفی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان تهران، سازمان تحقیقات، آموزش و ترویج کشاورزی، ورامین، ایران
مصطفی عبادی: استادیار، گروه زیست‌شناسی، واحد دامغان، دانشگاه آزاد اسلامی، دامغان، ایران

خلاصه

پژوهش حاضر، به منظور بررسی تنوع ژنتیکی چند رقم و هیبرید مختلف پنبه شامل *Gossypium hirsutum* cultivar Varamin، *G. hirsutum* cultivar Varamin × *G. barbadense* cultivar T14، *G. herbaceum* cultivar Arya، *G. barbadense* cultivar T14 (V×T14) و هیبرید *G. hirsutum* cultivar Varamin × *G. herbaceum* cultivar Arya (V×A) با استفاده از نشانگر مولکولی ISSR انجام شد. در این پژوهش، از چهار آغازگر استفاده شده، تعداد ۶۰ باند ایجاد گردید که از این تعداد، ۵۷ باند، پلی‌مورفیمی را نشان دادند. نتایج حاصله همچنین درصد بالایی از تنوع را در ارقام مورد مطالعه نشان داد. بالاترین درصد پلی‌مورفیمی در آغازگر ISSR4 با توالی ACACACACACACT (۱۰۰٪) و کمترین درصد پلی‌مورفیمی در آغازگر ISSR1 با توالی AGAGAGAGAGAGAGC دیده شد (۹۲٪). آنالیز خوشه‌ای، سه گروه بزرگ را نشان داد. گروه اول شامل ارقام آریا و ورامین، گروه دوم شامل ارقام هیبریدی ورامین در آریا و T14 بود که این تشابه ژنتیکی بیانگر این بود که رقم T14 از رقم ورامین اشتقاق یافته و گروه سوم شامل ارقام هیبریدی ورامین در T14 بود. نتایج حاصل از این پژوهش نشان داد که نشانگر مولکولی ISSR می‌تواند به طور مؤثری در مطالعه تنوع ژنتیکی در گیاه پنبه مورد استفاده قرار گیرد.

واژه‌های کلیدی: پنیکیان، تتراپلوئید، تنوع ژنتیکی، دیپلوئید، رقم، *Gossypium*

Introduction

Knowledge of genetic diversity in cultivating crops plays an important role in promoting breeding programs, crop management, and conservation of genetic resources. In this concern, diverse attempts have been employed to develop novel biochemical and molecular procedures to explore genetic diversities among plant species (Semagn *et al.* 2006, Kumar *et al.* 2009, Idrees & Irshad 2014, Amom & Nongdam 2017, Nilkanta *et al.* 2017). Increasing production and improving the quality of crops and optimizing the use of genetic resources requires the collection, preservation, and evaluation of genetic material. Reproductive system, reproduction mechanism, the density of population, evolutionary agents, and gene flow are considered as diverse determining factors affecting genetic variation among plant species (Amom & Nongdam 2017). Taking progress in molecular science and technology into account, a plethora of molecular techniques have been employed to monitor underlying genetic variations in various plant species by utilization of diverse DNA-based markers independent of external cues. The utilization of plenty of DNA markers as significant molecular tools provides a great opportunity to monitor genetic variation among plant species. Sequence related amplified polymorphism (SRAP), single nucleotide polymorphism (SNP), inter-simple sequence repeats (ISSR), amplified fragment length polymorphism (AFLP), and inter-primer binding site (iPBS), and start codon targeted (SCoT) are known as more reliable advanced PCR-dependent polymorphic markers than the restriction fragment length polymorphism (RFLP) as a hybridization-based marker (Amom & Nongdam 2017). Vafaie-Tabar *et al.* (2004) used RAPD analysis to assess genetic diversity among Tetra and diploid cotton (*Gossypium hirsutum* L. and *G. arboreum* L.), and showed that, the inter-specific genetic relationships were related to their pedigree and tetraploids show much narrow genetic base than diploids cotton varieties. In plant-related studies, ISSR markers as PCR-based molecular tools displaying mostly dominant inheritance

profiles have been widely applied to figure out gene mapping, genetic variations, and varietal identification. Several lines of convincing reports confirmed the beneficial function of the ISSR technique to study genetic variation among plant species (Nilkanta *et al.* 2017).

Cotton is an important plant in the world grown for its oil and fibers which are widely used in the textile industry. Its cultivation has a long history in Iran and is one of the main crops in northeastern Iran. Cotton (*Gossypium* spp.) is a dicotyledonous plant of the *Malvaceae* family. This genus includes 49 species out of which, 18 species are found in North, South, and Central America, 14 species in northeast Africa and southwest Asia, and 17 variations in Australia (Wendel *et al.* 1992). Among these species, 44 species are diploid, whereas five species are tetraploid (Endrizzi *et al.* 1985). *G. herbaceum* L., *G. arboretum* L., *G. hirsutum* L., and *G. barbadense* L. are the dominant cultivating crops widely exploited in the textile industry (Wendel *et al.* 1992, Tohidfar *et al.* 2008). Among different cotton species, there are variations in terms of crop yield and resistance to pests. It has become evident that, diploid species are more resistant to pests while tetraploid species have a higher yield than the diploid (Endrizzi *et al.* 1985). In the world, the most dominant cotton species is tetraploid *G. hirsutum*, which is widely adapted to many areas and comprises almost 90% of the world's cotton production. Hybridization provides great opportunity to improve the genetic diversity in industrial crops, like cotton (Noormohammadi *et al.* 2013a). Vafaie-Tabar *et al.* (2014) reported successful generation and plant establishment of a triploid hybrid ($3x = 39$, AAD) between *G. hirsutum* ($4n = 2x = 52$, AADD) and *G. arboreum* ($2n = 2x = 26$, AA) by *in vitro* culture of hybrid embryos excised from field pollinated seeds. SSRs are known as a new molecular marker which use to evaluate the genetic diversity in the germplasm of cotton plants (Malik *et al.* 2014). There are many studies about utilizing the SSR markers for genetic variation. Liu & Wendel (2001) introduced double priming ISSR as an

informative protocol to characterize inter/intraspecific variations in cotton (Liu & Wendel 2001). Abdellatif & Soliman (2013) investigated variations in genetic traits and morphological indexes among 24 different genotypes of *G. barbadense* using several molecular markers, including SSR, EST, and ISSR among which the most informative markers were ISSR markers. It seems that, the molecular findings are more reliable than the morphological characteristics, mainly owing to the dynamic changes in morphological traits in response to environmental factors (Abdellatif & Soliman 2013). Noormohammadi *et al.* (2013a) also concluded that, ISSR is a more efficient procedure to identify polymorphism among the cotton genotypes when compared to other kinds of molecular markers. Multani & Lyon (1995) concluded that, closely related cultivars of *G. hirsutum* can be identified by specific molecular markers. Investigation on interspecific polymorphism among *G. hirsutum* and *G. barbadense* using SSRs markers revealed that, polymorphism was high between species, while it was low at the level of intra-species (Rungis *et al.* 2005). De Magalh *et al.* (2006) evaluated diversity between 52 *G. hirsutum* cultivars using 31 SSR primers and successfully detected 52 cultivars. Likewise, genetic diversity of 193 cotton cultivars (Fang *et al.* 2013), 50 Pakistani genotypes (Dahab *et al.* 2013), 56 sea-island cotton accessions (Wang *et al.* 2011), 19 Bt cotton genotypes (Ullah *et al.* 2012), and 24 cotton genotypes (Abdellatif & Soliman 2013) have been assessed using 448, 70, 237, 104, and 36 SSR markers,

respectively. Using SSR and EST-derived SSR markers, Noormohammadi *et al.* (2013b) studied a potential genetic variation among 21 cotton genotypes in Iran. SSRs are considered a useful tool for evaluating the genetic purity of the cotton hybrids and hybrid identification. Successful breeding programs mostly are related to the availability of the genetic variation in germplasm accessions (Malik *et al.* 2014).

It is important to note that, the rationale for selecting the samples studied in this study is the commercial importance of these hybrids in Iran. The current study was, therefore, conducted to explore genetic variations among several cotton species, including a hybrid of *G. hirsutum* cultivar Varamin × *G. barbadense* cultivar T14 (V×T14), a hybrid of *G. hirsutum* cultivar Varamin × *G. herbaceum* cultivar Arya (V×A), and their parents using ISSR as important molecular markers.

Materials and Methods

In the present study, genetic diversities were evaluated among four different diploid, triploid, and tetraploid cotton genotypes, including *G. hirsutum* cultivar Varamin, *G. barbadense* cultivar T14, *G. herbaceum* cultivar Arya, and hybrid of *G. hirsutum* cultivar Varamin × *G. barbadense* cultivar T14 (V×T14), and *G. hirsutum* cultivar Varamin × *G. herbaceum* cultivar Arya (Table 1). All of these cultivars were supplied from the Varamin Cotton Research Institute (Varamin, Iran).

Table 1. The studied plant species provided by the Varamin Cotton Research Institute (Varamin, Iran)

Taxon/Cultivar	Characteristic
<i>Gossypium hirsutum</i> cultivar Varamin	Tetraploid
<i>G. barbadense</i> cultivar T14	Tetraploid
<i>G. herbaceum</i> cultivar Arya	Diploid
<i>G. hirsutum</i> cultivar Varamin × <i>G. barbadense</i> cultivar T14	Tetraploid
<i>G. hirsutum</i> cultivar Varamin × <i>G. herbaceum</i> cultivar Arya	Triploid

- DNA extraction and ISSR analysis

According to the CTAB method (KitCAT.#PT71817), DNA was extracted from liquid nitrogen well-grounded leaves. Briefly, 1400 μ l CTAB buffer and 10 μ l proteinase K were added to the samples, kept under 65 °C for 45 min, and finally microfused. Next, a mixture of phenol: chloroform: isoamyl alcohol (1:24:25) was added to the supernatant and followed by microfusion process. From the three formed phases, the supernatant containing DNA was carefully separated. DNA purity was evaluated based on the ratio of absorbance at 260 to 280 nm. PCR amplifications were

carried out using a DNA thermal cycler (Applied Biosystems Veriti). The sequences of four applied primers are presented in table 2. Reaction mixture contained 1 μ L primer, 1 μ L genomic DNA, 0.75 μ L MgCl₂, 2.5 μ L PCR buffer, 0.5 μ l dNTPs, 0.2 μ L Taq DNA polymerase, and 18.05 μ L double-distilled H₂O. PCR amplifications were performed under programming cycles of 95 °C for 5 min, 94 °C for 7 s, 36 °C for 45 s, 72 °C for 1 min, and 72 °C for 7 min. The PCR products were loaded on 1.5% agarose gel and electrophoresed.

Table 2. Sequences of the applied ISSR markers

Marker	Sequence
ISSR 1	AGAGAGAGAGAGAGAGC
ISSR 2	GAGAGAGAGAGAGAGAC
ISSR 3	GAGAGAGAGAGAGAGAA
ISSR 4	ACACACACACACACT

- Data analysis

Genotypes were compared by ISSR based on the presence or absence of amplified fragments (Fig. 1). PCR bands were evaluated using UPGMA and DARwin Ver.

6 software. The molecular data were analysed using NTSRS-PC2.2 software. Genetic similarities were calculated by Jaccard & Nei's (1972) genetic similarity coefficient.

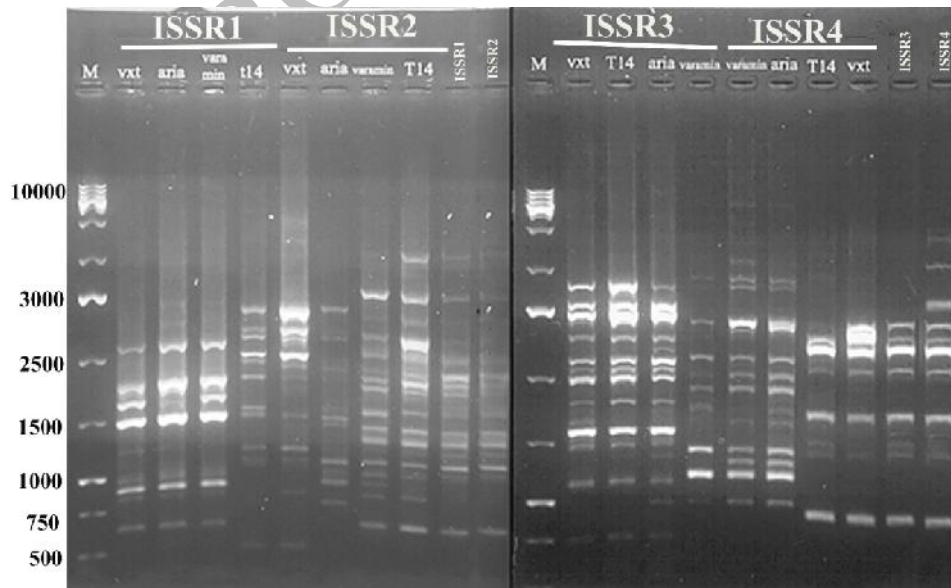


Fig. 1. DNA amplification profile in five cotton genotypes, with four ISSR primers.

Results and Discussion

In the present study, molecular markers were used to detect the genetic similarity of cotton in Iran. The four chosen ISSR primers produced different numbers of DNA fragments (Table 3). In ISSR1, a total of 33 fragments were detected among which, 13 (92.8%) fragments were polymorphic. A total of 106 fragments were produced in ISSR2 among which, 14 fragments were found amplified having 13 (92.8%) polymorphic bands. In ISSR3, the total produced band was 41 of which, 17 (94.1%) amplified fragments were polymorphic. In ISSR4, the total number of examined bands was 36 among which, 15 were amplified fragments and the number of polymorphs was 100 resulting in 100% polymorphism (Table 3).

Evaluating H (expected heterozygosity), PIC (polymorphism information content), E (multiple effective coefficients), H_{av} (heterozygous mean), MI (marker index), D (differentiation coefficient), and R (solubility coefficient) indices showed that, there were no

significant differences between the four primers regarding H parameter. ISSR2 and ISSR4 did not differ in H. However, the ISSR1 and ISSR3 showed the lowest and highest numbers, respectively (Table 4). PIC (Polymorphism Information Content) varied from 0.26 (ISSR1) to 0.29 (ISSR3) (Table 4). E (multiple effective factors) ranged from 6.75 (ISSR1) to 8 (ISSR3) (Table 4). MI (marker index) was between 0.01 for ISSR1 primer up to 0.02 for ISSR3 primer (Table 4). The range of R (solubility coefficient) was between 3.5 for ISSR2 up to 12 for ISSR3 (Table 4). Take D index into account, there was no significant difference in the four primers (Table 4). In this study, five samples were investigated, of which three were parental and two hybrid. The genetic relationships were estimated from the markers data based on Jaccard's similarity index. According to the results, genetic similarity ranged from 0.18 (low similarity) between sample Arya and V×T14 up to 0.452 (high similarity) between sample V×A and T14 (Table 5).

Table 3. Name and details of used ISSR primers

Marker	Sequence	No. of amplified fragments	No. of polymorphic fragments	Polymorphism %age
ISSR1	AGAGAGAGAGAGAGGC	14	13	92.8
ISSR2	GAGAGAGAGAGAGAC	14	13	92.8
ISSR3	GAGAGAGAGAGAGAA	17	16	94.1
ISSR4	ACACACACACACACT	15	15	100

Table 4. Genetic parameters in cotton genotypes are based on four ISSR primers [Indexes: H (expected heterozygosity), PIC (polymorphism information content), E (multiple effective coefficients), H_{av} (heterozygous mean), MI (marker index), D (differentiation coefficient), and R (solubility coefficient)]

Marker	BN	TAB	NPB	PPB	H	PIC	E	H _{av}	MI	D	R
ISSR1	33	14	13	92.8	0.31133	0.262864	6.75	0.002224	0.01501	0.963926	9.5
ISSR2	106	14	13	92.8	0.3448	0.285354	7.75	0.002463	0.019087	0.95221	6.5
ISSR3	41	17	16	94.1	0.35265	0.290471	8	0.002519	0.020152	0.949024	12
ISSR4	36	15	15	100	0.3448	0.285354	7.75	0.002463	0.019087	0.95221	14.5

Table 5. Jaccard's Similarity Index

Taxon/Cultivar	V×A	Arya	Varamin	T14	V×T14
V×A	1	0.428	0.255	0.452	0.266
Arya	0.428	1	0.418	0.285	0.18
Varamin	0.255	0.418	1	0.265	0.208
T14	0.452	0.285	0.265	1	0.363
V×T14	0.266	0.18	0.208	0.363	1



Fig. 2. UPGMA clustering based on Nei's Genetic Distance in five cotton genotypes.

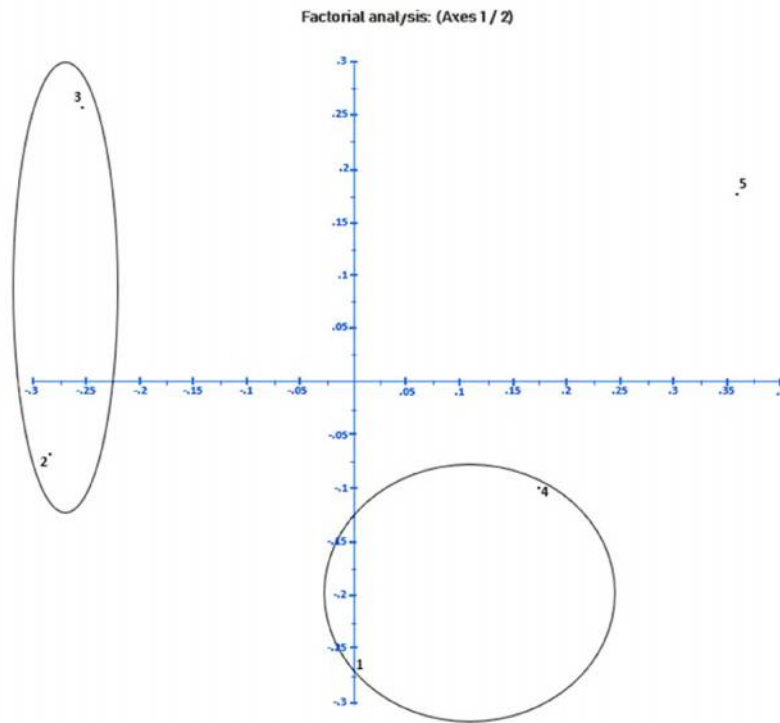


Fig. 3. Two-dimensional plot of principal component analysis of five cotton genotypes: (1) V×A, (2) Arya, (3) Varamin, (4) T14, (5) V×T14.

Cluster analysis showed three major groups (Fig. 2). The first group consisted of Arya and Varamin. The second group was comprised of V×A, T14 which showed a high genetic similarity indicating that, T14 originates from Varamin. The third one contained V×T14. These results indicate the high genetic similarity among the two tetraploid and diploid groups. PCoA plot of cotton genotypes completely supported the grouping recorded by clustering and exhibited genetic similarity among

V×A, T14, and distinctness of V×T14 (Fig. 3). The finding is completely inconsistent with the results of Dongre *et al.* (2007) who used the ISSR marker to investigate the diversity of cotton variations. Dongre *et al.* (2007) provided DNA profiles of 19 genotypes of diploid and tetraploid cotton using two marker systems, including ISSR and microsatellite. Their findings confirmed the existence of genetic variation among the studied genotypes of *G. arboreum* and

G. hirsutum. Qureshi *et al.* (2004) utilized EST-SSR primers to show diversity among *G. hirsutum* and *G. barbadense* cultivars. Several lines of SSR studies identified genetic diversity among various cotton species (Multani & Lyon 1995, Liu & Wendel 2001, De Magalh *et al.* 2006, Wang *et al.* 2011, Ullah *et al.* 2012, Abdellatif & Soliman 2013, Dahab *et al.* 2013, Fang *et al.* 2013, Noormohammadi *et al.* 2013a,b).

In a supplementary study, a karyotyping study was performed to detect potential variations and chromosomal abnormalities in hybrids relative to their parents. The ratio of the arms, the position of the centromere, and the number of satellite chromosomes were monitored. This study revealed that, the Arya cultivar has thirteen pairs of chromosomes among which the satellites are located on chromosomes of ten (the longest length) and thirteen (the shortest length) (Fig. 4). The highest amounts of L/S ratio were found in the chromosomes containing satellites. The L/S ratio for chromosome 10 was 4.89 which is the same among both parent and hybrid samples. While the Varamin cultivar has 26 pairs of chromosomes, the satellites are located on chromosomes 15 and 2. Moreover, chromosome 22 showed the highest L/S ratio (2.88 μm) (Fig. 5). According to the chromosomal karyotype, the triploid cultivar V \times A hybrid displays abnormality in terms of chromosomal traits (shape, length, ratio, and satellites) when compared to the parents (Varamin and Arya). We observed 39 chromosomes ($2n = 3x = 39$) exhibiting several abnormal couplings among homologous chromosomes in comparison with the parents (Fig. 6). Interestingly, no satellite chromosomes were found in this hybrid. In this hybrid, the highest L/S ratio (3.15 μm) was related to the 30th chromosome. Among the studied groups, the V \times A hybrid had the lowest L/S ratio in the chromosome of 39. As another evidence, the ratio of a long arm to a short arm in this hybrid is different from

the parents. This is most likely related to the chromosome pairing patterns, chromosomal deletion/insertion events, and possibly crossing over phenomenon. The observed results can be, therefore, explained by the variation that occurs in this hybrid (V \times A). T14 cultivar possesses 26 pairs of chromosomes that, the satellites are located on chromosomes of 14 and 20 (Fig. 7). In this cultivar, chromosome 7 had the highest L/S ratio. Likewise, the tetraploid cultivar V \times T14 also displays a differential chromosomal pattern in terms of chromosome traits when compared to the parents. In this hybrid, 52 chromosomes ($48 = 4X = 52$) are characterized during the metaphase stage of the cell cycle. In the V \times T14 hybrid, the chromosomes of 1 and 52, respectively, had the highest and lowest length among the samples studied (Fig. 8). However, a comparison of ideograms revealed that, the V \times T14 hybrid exhibited a more organized arrangement than the V \times A hybrid. This can be explained by the higher similarity between the Varamin and T14 parents than the Varamin and Arya. With a similar trend to the V \times A hybrid, the deletion process of chromosomes containing satellites was observed in the V \times T14 hybrid. Our results are in line with findings of Noormohammadi *et al.* (2013b) who reported allelic similarity/gene exchange between *G. arboretum* and *G. herbaceum* genotypes (Noormohammadi *et al.* 2013b).

Taken collective, this study displayed the efficiency of the ISSR method to identify genetic diversity among various cotton species. This study, provides molecular evidence on how hybridization can be associated with substantial genetic variations, including the chromosome pairing patterns and chromosomal deletion/insertion events relative to the parents. The obtained results are worthy of characterizing the genotypes for using in hybridization program to achieve a higher level of genetic variation in cotton.

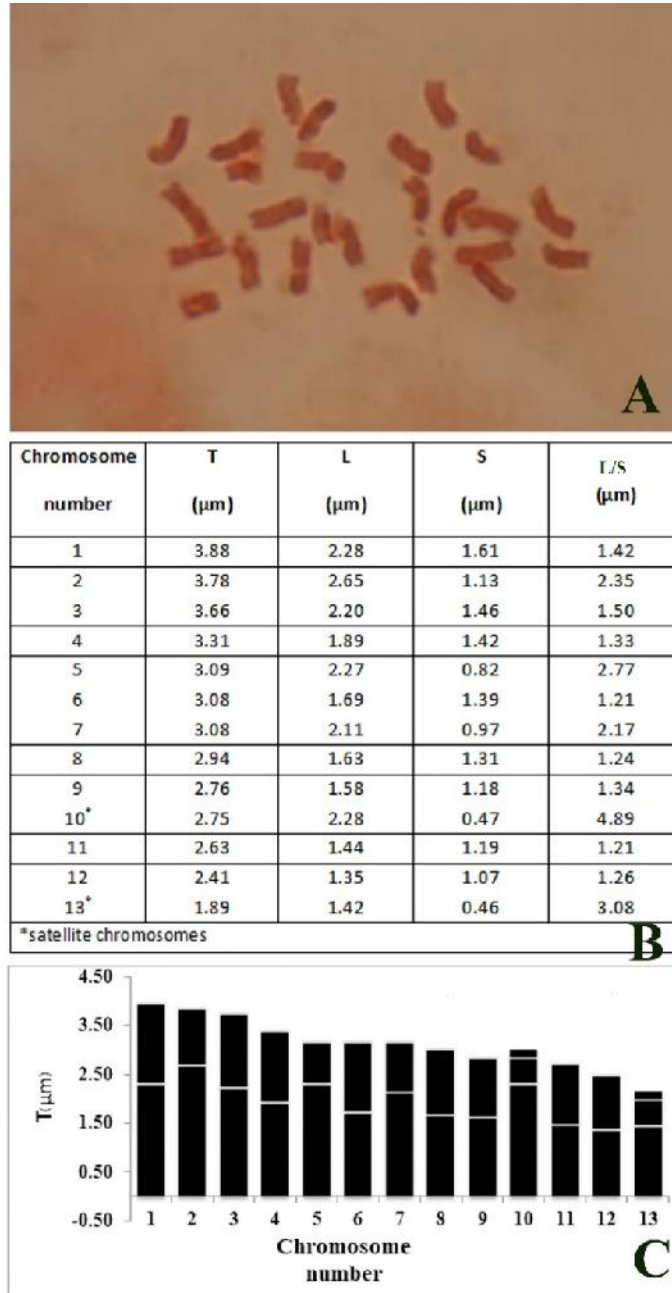


Fig. 4. A. Karyotype, B. Chromosomal characteristics, C. ideogram in Arya cultivar. T: total chromosome length, L: length of long arm, S: length of small arm, L/S: length of a long arm to length of small arm.

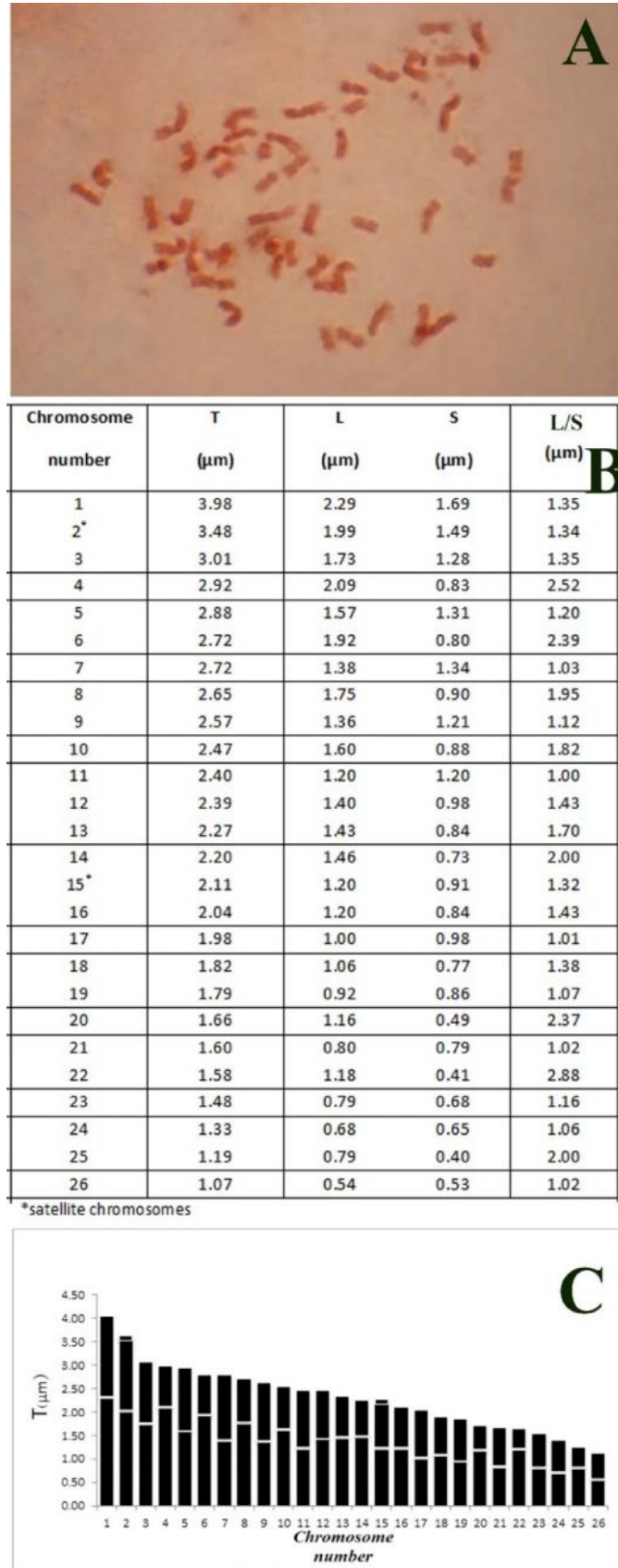


Fig. 5. A. Karyotype, B. Chromosomal characteristics, C. Ideogram in Varamin cultivar. T: total chromosome length, L: length of long arm, S: length of small arm, L/S: length of a long arm to length of small arm.

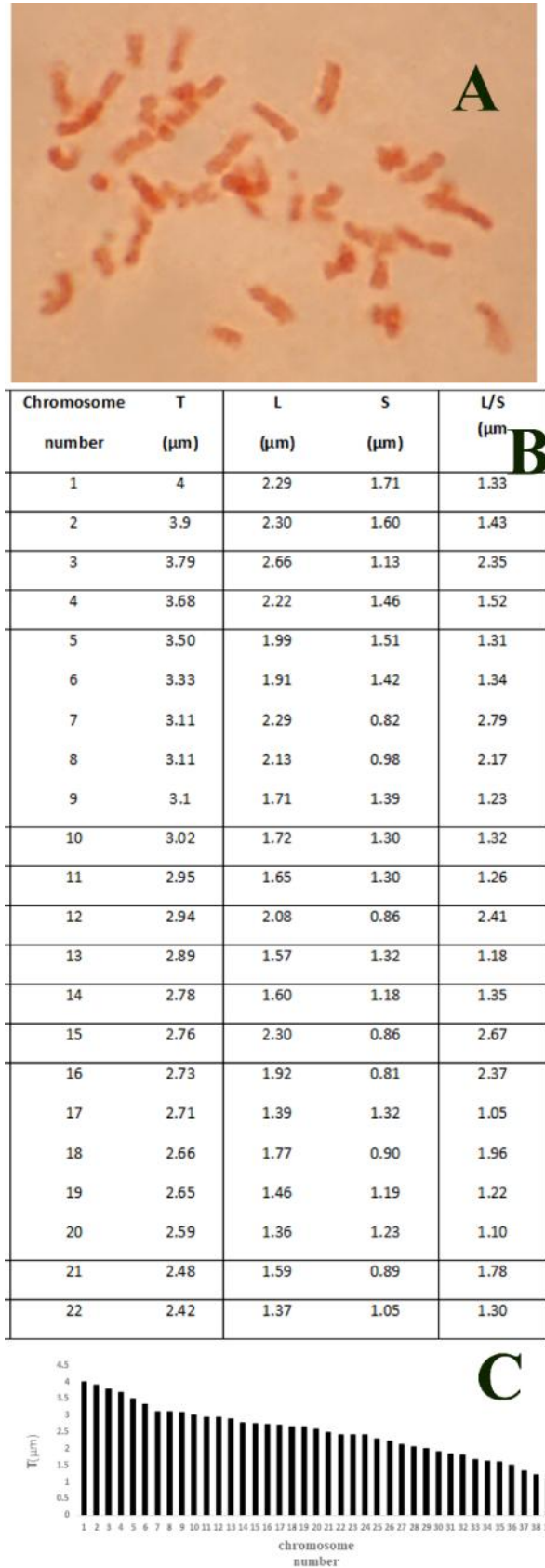


Fig. 6. A. Karyotype, B. Chromosomal characteristics, C. Ideogram in VxA hybrid. T: total chromosome length, L: length of long arm, S: length of small arm, L/S: length of a long arm to length of small arm.

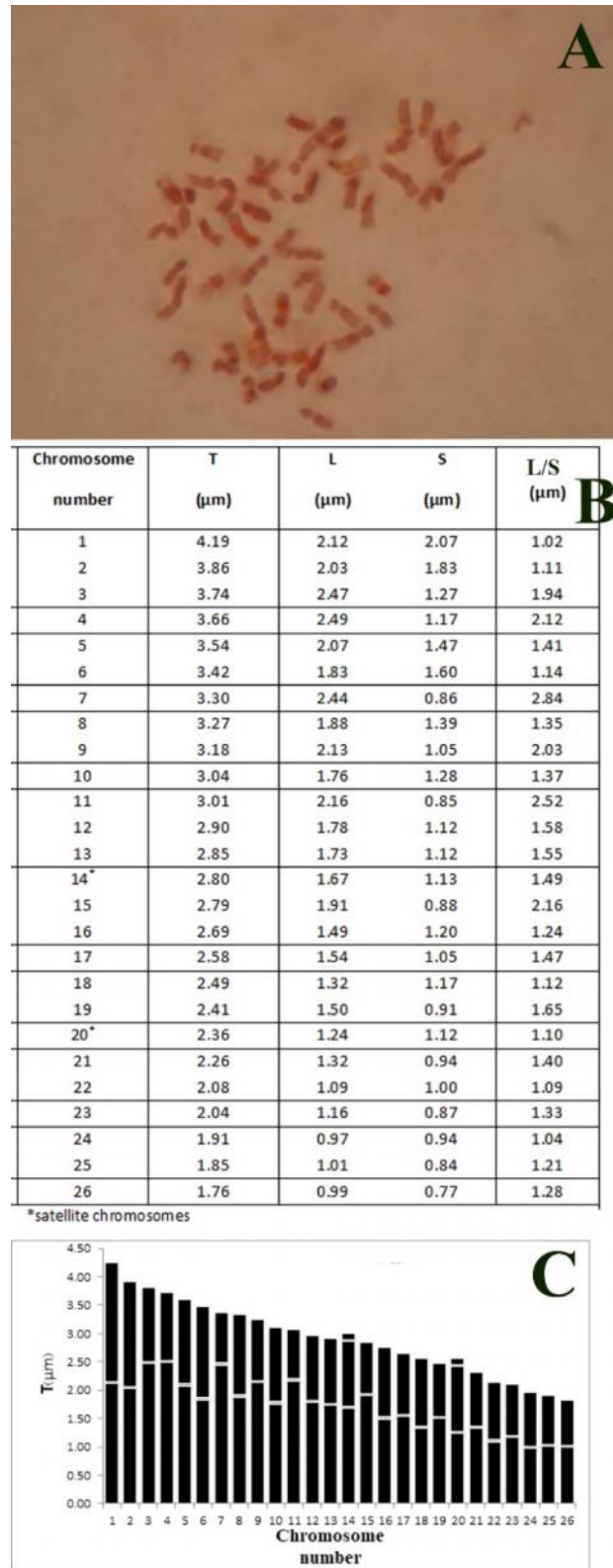


Fig. 7. A. Karyotype, B. Chromosomal characteristics, C. Ideogram in T14 cultivar. T: total chromosome length, L: length of long arm, S: length of small arm, L/S: length of a long arm to length of small arm.

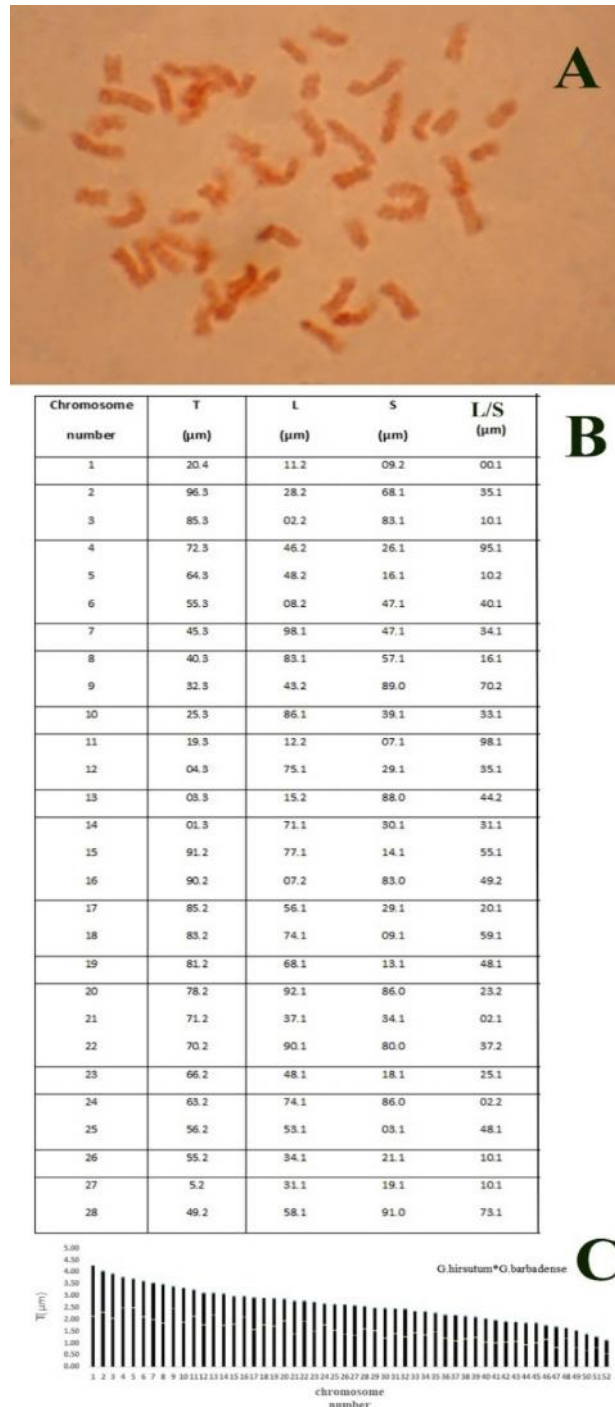


Fig. 8. A. Karyotype, B. Chromosomal characteristics, C. Ideogram in V×T14 hybrid. T: total chromosome length, L: length of long arm, S: length of small arm, L/S: length of a long arm to length of small arm.

Acknowledgments

Authors would like to express their appreciation to the faculty of the Research Assistant of the Science and Research Branch, Islamic Azad University (Tehran,

Iran) for the allocation of the necessary funds, and also to the Cotton Research Department, Tehran Agricultural and Natural Resources Research Center (AREEO), Varamin, Iran for providing the plant materials.

References

- Abdellatif, K.F. & Soliman, Y.A. 2013. Genetic relationships of cotton (*Gossypium barbadense* L.) genotypes as studied by morphological and molecular markers. *African Journal of Biotechnology* 12(30): 4736–4746.
- Amom, T. & Nongdam, P. 2017. The use of molecular marker methods in plants, a review. *International Journal of Current Research and Review* 9: 1–7.
- Bertini, H.C., Schuster, I., Sedyama, T., Barros, E.G. & Moreira, M.A. 2006. Characterization and genetic diversity analysis of cotton cultivars using microsatellites. *Genetics and Molecular Biology* 29(2): 321–329.
- Dahab, A.A., Saeed, M. & Mohamed, B.B. 2013. Genetic diversity assessment of cotton (*Gossypium Hirsutum*) genotypes from Pakistan using simple sequence repeat marker. *Australian Journal of Crop Science* 7: 261–267.
- De Magalh Bertini, C.H., Schuster, I., Sedyama, T., Barros, E.G.D. & Moreira, M.A. 2006. Characterization and genetic diversity analysis of cotton cultivars using microsatellites. *Genetics and Molecular Biology* 29(2): 321–329.
- Dongre, A.B., Bhandarkar, M. & Banerjee, Sh. 2007. Genetic diversity in tetraploid and diploid (*Gossypium* spp.) using ISSR and microsatellite DNA markers. *Indian Journal of Biotechnology* 6: 349–353.
- Endrizzi, D., Day, T. & Gathman, A.C. 1985. Centromer orientation of quadrivalents of heterozygous translocations and an autopolyploid of *G. hirsutum*. *Genetics* 105: 723–731.
- Fang, D.D., Hinze, L.L., Percy, R.G., Li, P., Deng, D. & Thyssen, G.A. 2013. Microsatellite-based genome-wide analysis of genetic diversity and linkage disequilibrium in upland cotton (*Gossypium hirsutum* L.) cultivars from major cotton-growing countries. *Euphytica* 191: 391–401.
- Idrees, M.U. & Irshad, M.U. 2014. Molecular markers in plants for analysis of genetic diversity. *European Academic Research* 2: 1513–1540.
- Jaccard, P. 1908. Nouvelles Recherches Sur la Distribution Florale. *Bulletin de la Société vaudoise des sciences naturelles* 44: 2423–2470.
- Kumar, P., Gupta, V.K., Misra, A.K., Modi, D.R. & Pandey, B.K. 2009. Potential of molecular markers in plant biotechnology. *Plant Omics Journal* 2: 141–157.
- Liu, B. & Wendel, J.F. 2001. Intersimple sequence repeat (ISSR) polymorphisms as a genetic marker system in cotton. *Molecular Ecology Notes* 1(3): 205–208.
- Malik, W., Ashraf, J., Iqbal, M.Z., Ali Khan, A., Qayyum, A., Ali Abid, M., Noor, E., Qadir, A.M. & Abbasi, G. 2014. Molecular markers and cotton genetic improvement: current status and future prospects. *The Scientific World Journal* <http://dx.doi.org/10.1155/2014/607091>.
- Multani, D.S. & Lyon, B.R. 1995. Genetic fingerprinting of Australian cotton cultivars with RAPD markers. *Genome* 38(5): 1005–1008.
- Nilkanta, H., Amom, T., Tikendra, L., Rahaman, H. & Nongdam, P. 2017. ISSR marker based population genetic study of *Melocanna baccifera* (Roxb.) Kurz: A commercially important bamboo of Manipur, North-East India. *Scientifica* 17–29.
- Noormohammadi, Z., Farahani, Y.H.A., Sheidai, M., Ghasemzadeh-Baraki, S. & Alishah, O. 2013a. Genetic diversity analysis in Opal cotton hybrids based on SSR, ISSR, and RAPD markers. *Genetics and Molecular Research* 12(1): 256–269.
- Noormohammadi, Z., Rahnama, A. & Sheidai, M. 2013b. EST-SSR and SSR analyses of genetic diversity in diploid cotton genotypes from Iran. *The Nucleus* 56: 171–188.
- Qureshi, S.N., Saha, S., Kantety, R.V. & Jenkins, J.N. 2004. EST-SSR: A new class of genetic markers in cotton. *Journal of Cotton Science* 8: 112–123.

- Rungis, D., Llewellyn, D., Dennis, E.S. & Lyon, B.R. 2005. Simple sequence repeat (SSR) markers reveal low levels of polymorphism between cotton (*Gossypium hirsutum* L.) cultivars. Australian Journal of Agricultural Research 56: 301–307.
- Semagn, K., Bjørnstad, Å. & Ndjiondjop, M.N. 2006. An overview of molecular marker methods for plants. African Journal of Biotechnology 5: 25–34.
- Tohidfar, M.B., Ghareyazie, M., Mousavi, Sh., Yazdani, M. & Gholabchin, R. 2008. *Agrobacterium* mediated transformation of cotton (*Gossypium hirsutum*) using a synthetic cry1 Ab gene for enhanced resistance against *Heliothis armigera*. Iranian Journal of Biotechnology 6(3): 164–173.
- Ullah, A., Iram, M.Z., Iqbal, M., Nawaz, S., Hasni, M. & Jamil, S. 2012. Genetic diversity analysis of Bt cotton genotypes in Pakistan using simple sequence repeat markers. Genetics and Molecular Research 11: 597–605.
- Vafaie-Tabar M.V., Chandrashekar, S., Rana M.K. & Bhat, K.V. 2004. RAPD analysis of genetic diversity in Indian tetraploid and diploid cotton (*Gossypium* sp.). Journal of Plant Biochemistry and Biotechnology 13: 81–84.
- Vafaie-Tabar, M.V., Bhat, S. & Chandrashekar, S. 2014. Production of an interspecific triploid hybrid between *Gossypium hirsutum* and *G. arboreum* by embryo rescue. Journal of Plant Physiology and Breeding 4(1): 63–74.
- Wang, X.Q., Feng, C.H., Lin, Z. & Zhang, X. 2011. Genetic diversity of sea-island cotton (*Gossypium barbadense*) revealed by mapped SSRs. Genetics and Molecular Research 10(4): 3620–3631.
- Wendel, J.F., Brubaker, C.L. & Percival, A.E. 1992. Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. American Journal of Botany 79: 1291–1310.