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Full Length Research Paper

Intragenomic diversity and geographical adaptability of diploid cotton species revealed by cytogenetic studies

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Cotton is one of the most important crops in Iran, and is cultivated in different regions of the country. *Gossypium herbaceum* is one of the A-genome cottons, which is a potentially important genetic resource for cotton breeding programs. Collecting native cultivars of this species growing in different regions is a vital step in broadening variability of the gene pool. The *G. herbaceum* is one of the two cultivated species under cultivation in Iran, which is specifically adapted to a given environment and includes more than 40 ecotypes, named as landrace cottons. The present paper reports the intragenomic characteristics analysis of 42 *G. herbaceum* cultivars in the cotton genebank using cytological methods. The karyological studies showed variations within the species in the size of chromosome, chromosome volume and karyotype formulae. All cultivars possessed 2n=26 chromosome, but varied with regard to number of SAT-chromosomes (ranging from 1 to 3) and the chromosomes carrying secondary constructions. Karyotypes were of symmetrical type, having small chromosomes. Analysis of variance revealed significant differences between the cultivars as well as the chromosomes. Cluster analysis could group the cultivars in four distinct clusters. The present study indicates genomic differences among diploid *G. herbaceum* cultivars, which can be used in cotton hybridization programs in Iran or other countries.

Key words: *Gossypium herbaceum*, Intragenomic diversity, Adaptability, Karyotype.

INTRODUCTION

Variation in chromosome number and karyotype has proved to be an important source of information for understanding plant evolution (Stebbins, 1971; Raven, 1975). Cotton belongs to *Gossypium* genus and is accounted as a genetic resource in domesticated and wild forms. Amongst 50 species identified and described in *Gossypium,* 44 are diploid (2n=2x=26) and the remaining are allotetraploids (2n=4x=52). Amongst the diploids, *G. herbaceum* and *G. arboreum* have been domesticated for cultivation. These cultivated species embody considerable genetic diversity as they are acclimatized to coastal (Fryxell, 1992), as well as desert ecosystem (Stewart, 1995). However, the genetic pattern of the polyploid cotton differs in some respects from that of the diploid (Zhou, 2003).

Genetic diversity provides a buffer against adverse effects of abiotic stresses and the genetic diversity among all available cultivars has been generally higher than the diversity that exists among widely grown cultivars, because producers tend to plant a few preferred cultivars (Khadi et al., 2002). Hence, collection, maintenance, documentation, characterization and utilization of genetic resources make important principles of any crop breeding program and cotton is not exception (Khadi et al., 2002).

Gossypium herbaceum is one of the A-genome cottons, which is a potentially important genetic resource for cotton breeding programs (Stantone et al., 1994). Use of

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of A-genome cotton as a source of genetic diversity for gaining stress tolerance, fiber quality and earlier maturity has suggested by several workers (Stewart, 1992). Collecting native cultivar of this species growing in different regions is a vital step in broadening variability of the gene pool. Several studies have indicated the presence of infraspecific variations in *G. herbaceum* (Wendel et al., 1992, Stanton et al., 1994).

The *Gossypium hirsutum* and *G. herbaceum* are two cultivated species cultivated in Iran. *G. herbaceum* is accounted as landrace cotton which is specifically adapted to a given environment and includes more than 40 ecotypes, which the majority of varieties was collected at the altitude range of 10-500 m, and is grown at 6-10% of the cultivation area in Iran (Sheidai and Alishah, 1998).

The landraces are traditionally grown under marginal conditions such as poor soil moisture, low fertility, hot windstorm, and the farmers do not use any inputs or improved management practices (Halila et al., 1990). Moreover, *G. herbaceum* cultivars grown in Iran show disease tolerance and, hence such trait can be considered in crossing programs. Data from morphological characters along with cluster analysis divided different cultivars of landrace cotton in 5 to 6 groups (Sheidai et al., 1996).

Studies on cytogenetics, chromosome structure, behaviour and manipulation in plants are well documented (Karpenchenko, 1925; Sarbhoy, 1977; Okoli and Olorode, 1983; Obute, 2001; Obute et al., 2006). The usefulness of information from such studies in the understanding of phylogenetic relationships, genetic mapping and breeding studies has been very significant (Okoli, 1983; Anamthawat and Heslop, 1993; Hartwell et al., 2000; Kurata et al., 2002). Muravenko et al. (1998) suggested that the ancestral cotton genome contained 7 homologous pairs of chromosomes. They expressed that image-analysis technique for identification and quantitative analysis of chromosomes, especially with regard to small-chromosome species, is very feasible.

Although there are good collections of *G. herbaceum* cultivars in the Iran National Genebank (stored in cold room) and Iran Cotton Research Institute (ICRI) (fresh seed stored), no information is available on their cytogenetical characteristics. Therefore, one the most important of the objectives in this report was to study for first the cytogenetical characteristics of Iranian collection of *G. herbaceum* cultivars.

MATERIAL AND METHODS

Forty-two *G. herbaceum* cultivars available in cotton research institute were used in this study. Seeds collected from these plants after removing the linters were germinated in Petri dish, and freshly grown roots were used for karyological preparations. After pretreatment and fixation, root tips were stained using 2% acetic-orcein. Genomic characters were compared using total form percentage, ratio of longest to shortest chromosome (Verma, 1980), coefficient of variation (Verma, 1980), S%, difference of the range of relative length (D.R.L.) (Gennur et al., 1988) and total volume. Chromosome types were determined according to Levan et al (1964). After analysis of variance the genotypes were grouped on the base of karyological data using cluster analysis (UPGMA method) (Sheidai et al., 1996).

RESULTS

During the survey, 42 cultivars of *G. herbacum* were studied karyologically and the different characteristics were recorded. Cultivars and their genome details are represented in Table 1. ANOVA based on factorial analysis of karyotypic (Figure 1) data showed significant differences among the *G. herbaceum* cultivars in the size of chromosome (Table 2). The genome details in different cultivars indicated there were also variations in the sattelite number, total length of chromosome, long and short arm length, chromosome volume etc. Total chromosome length ranged from 23.83 µm in Mahallat landrace to 46.46 µm in Sabzevar 60-1 landrace, and average of chromosome length ranged from 1.83 µm (in Mahallat landrace) to 3.57 µm (in Sabzevar 60-1 landrace). The longest chromosome length ranged between 2.4 - 5.03 µm, and the shortest chromosome length ranged from 1.2 to 2.23 µm. Chromosome volume ranged from 4.37 μm^3 (Mahallat landrace) to 9.32 μm^3 (in Mehrize Bah. and Sabzevar 60-1).

Pearson coefficient for total length of the chromosomes was 0.95, but this value was reduced for the length of the long arms (0.72-0.96), length of short arms (-0.01-0.61) and L/S ratio (0.31-0.96). The karyotype formulae of different cultivars are represented in Table 1. Almost all the chromosomes were of “m” type, except for 1 or 3 chromosomes which were of “M” type (Figure 1). All the cultivars possessed 2n=26 chromosome, but varied with regard to number of SAT-chromosome (ranging from 1 to 3) and the chromosomes carrying secondary constructions. The SAT size varied from 0.27 µm in Qom landrace to 1 µm in colored lint landrace. Coefficient of variation (C.V.), T.F% and D.R.L. showed that the *G. herbaceum* ecotypes had symmetrical karyotypes with a small size of chromosome. Marvest Mehriz (red boll) and Aria landrace were more symmetrical than Mahallat and Marvest Mehriz (green and hairiness plant).

Average of genomic characteristics is showed in Table 3. As seen in the table, mean of chromosome length (X), different relative length (D.R.L.), total volume (T.V.) and number of satellites (No.SAT) were 2.65, 6.23, 7.08 and 1.88, respectively. Dendrogram produced from UPGMA cluster analysis is represented in Figure 2. According to cluster analysis and cutting dendrogram in a single distance coefficient (distance = ~7), studied cotton varieties divided into four clusters. Eighteen cultivars were presented in the first cluster, 13 cultivars in the second, 8 in the third, 3 in the forth cluster.

DISCUSSION

In this study we could distinguish different diploid cottons

Table 1. Cultivars and their genome details.

Cultivar	T.L	L	S	L/S	TF%	C.V	S%	X	D.R.L	T.V	Genome formula	SAT-Chr.
Kerman	31.63	3.53	1.54	2.3	44.0	22.0	51.0	2.3	5.2	6.5	1M+l2m	1
Kashan l2	30.65	3.27	1.67	2.0	41.7	20.0	43.6	2.4	6.3	5.8	3M+10m	1
Qoml (R-G)	28.89	3.26	1.33	2.4	40.9	23.0	40.8	2.2	6.7	5.4	13m	1, 3
Giroft	28.31	2.97	1.42	2.1	41.5	19.3	47.0	2.1	5.5	5.1	1M+l2m	1, 2
Garmsar 60	46.16	4.99	2.23	2.2	39.7	21.7	42.7	3.5	5.9	8.3	13m	1, 2, 3
Lasjard	34.65	3.75	1.58	2.3	43.3	22.1	42.1	2.6	6.2	6.8	13m	1, 2
Kerman B.	35.23	3.80	1.72	2.2	41.2	22.0	45.2	2.7	5.9	6.5	13m	1, 2, 3
Neiriz R.	36.10	4.31	1.73	2.5	42.5	26.1	40.1	2.7	7.1	6.6	13m	1, 8
Qom 52	37.44	4.81	1.76	2.7	42.9	30.1	36.4	2.8	8.1	7.0	13m	1, 3
Neiriz GAZ	35.82	4.26	1.58	2.7	38.7	28.5	37.0	2.7	7.4	6.6	13m	1, 2
Shahrood	38.10	4.30	1.66	2.6	40.0	26.0	38.6	2.9	6.0	7.2	13m	2, 4
Damghan	36.17	4.06	1.60	2.4	39.7	23.8	40.0	2.7	6.8	7.2	13m	1, 8
Shahreza	32.03	3.55	1.50	2.3	41.4	23.0	43.7	2.4	6.3	6.7	13m	1, 3
Neishaboor	37.42	4.28	1.67	2.5	39.6	26.0	39.1	2.8	6.9	8.2	13m	1, 4
Ardekan 1	34.68	3.73	1.64	2.2	40.3	22.0	43.0	2.6	6.0	7.0	13m	3, 4
Kashmar	37.20	3.82	1.91	2.0	41.3	19.0	50.0	2.8	5.1	7.9	13m	3, 4
Kerman GB.	37.61	3.97	1.82	2.1	41.4	21.8	45.8	2.9	5.7	7.9	13m	5, 7
Hashemabad	31.69	3.57	1.62	2.2	39.4	21.5	45.3	2.4	6.1	6.9	1M+l2m	1
Esfahan Ag.	41.13	4.49	1.76	2.5	41.5	23.7	39.1	3.1	6.6	9.1	1M+l2m	1, 4
Shooshtar	36.16	4.05	l.72	2.3	42.1	21.7	42.4	2.8	6.3	8.0	1M+l2m	1, 4
Gozagh	35.30	3.66	1.73	2.1	42.1	21.1	47.2	2.7	5.4	7.7	1M+l2m	1, 4
Mehriz Gud.sabz	30.78	3.45	1.50	2.3	39.8	25.4	43.3	2.3	6.3	6.3	1M+l2m	2, 4
Sabzevar l60.2	35.74	4.05	1.76	2.3	40.5	26.0	43.5	2.7	6.4	7.4	2M+IIm	1, 3
Marvast Sk	30.56	3.09	1.58	1.9	42.3	18.0	51.1	2.3	4.9	6.6	13m	3, 6
Rafsanjan	42.66	4.73	1.95	2.4	40.4	25.2	41.2	3.2	6.5	9.0	1M+l2m	1, 4, 8
Mehriz Bah.	42.77	4.96	1.56	3.1	40.6	28.3	31.4	3.3	7.9	9.3	13m	1, 5
Qom (Red)	30.35	3.24	1.60	2.0	40.2	21.5	49.3	2.3	5.4	6.1	2M+IIm	2
Mehriz Gud.Red	30.08	3.48	1.36	2.5	41.5	25.0	39.0	2.3	7.0	6.1	1M+l2m	1, 3
Ardekan.2	38.02	4.08	1.80	2.2	41.6	22.9	44.1	2.9	6.0	8.0	13m	1, 2
Garmsar	32.27	3.40	1.49	2.2	39.4	22.0	43.8	2.4	5.9	6.7	13m	1, 2
Colored lint	42.36	4.95	1.82	2.7	40.2	26.0	36.7	3.2	7.8	9.2	13m	1, 2, 6
Bandarabas	38.23	4.48	1.64	2.7	41.5	28.6	36.6	2.9	7.4	7.6	13m	3
Rafsanjan RB	34.50	3.90	1.72	2.2	43.1	24.0	44.0	2.6	6.3	6.8	1M+l2m	4
Rafsanjan RBs	35.14	4.13	1.59	2.6	41.7	27.6	38.5	2.7	7.2	7.1	13m	1, 2
Harat Mehriz	40.82	4.71	1..95	2.4	39.7	24.0	41.0	3.1	6.7	8.2	13m	1, 3
Qom white	40.18	4.79	1.82	2.6	39.2	27.0	38.0	3.1	7.4	7.9	13m	2, 5
Sabzevar60.1	46.46	5.03	2.01	2.5	41.3	24.8	39.9	3.5	6.5	9.3	13m	1, 4
Aria	27.80	2.71	1.71	1.5	43.2	16.2	63.0	2.1	3.6	5.2	2M+IIm	1
Mehriz (Red).	30.63	2.91	1.75	1.6	43.9	13.5	60.1	2.3	3.7	6.0	3M+10m	2
Mahallat	23.83	2.40	1..20	2.0	40.2	17.3	50.0	1.8	5.0	4.7	13m	3
Mehriz.Gud4	27.41	3.11	1..39	2.2	40.5	20.4	44.6	2.1	6.2	5.7	13m	1, 4
Mehriz 2	30.77	3.46	1..53	2.2	43.2	21.1	44.2	2.3	6.2	6.0	2M+IIm	1, 3

T.L = Total length, L = longest chromosome, S = shortest chr. TF% = total form percentage, C.V. = coefficient of variation, X = mean chr. Length, D.R.L. = different relative length, T.V. = total volume.

based on chromosome characteristics using cytogenetic approaches. As showed by karyotype analysis, significant differences were observed among the *G. herbaceum* cultivars in the size of chromosome, the satellite number, total length of chromosome, long and short arm length, chromosome volume etc. Coefficient analysis showed a high measure (0.95) for total length of chromosomes, indicating homogeneity of the group, but this value was

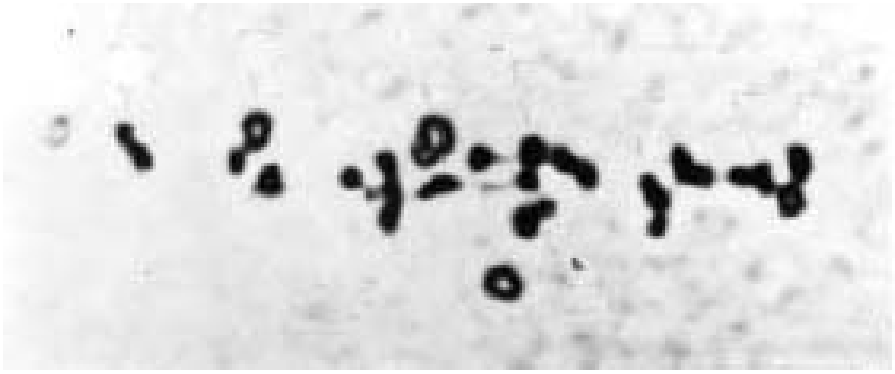

Figure 1. The karotype of one of the studied cultivars.

Table 2. ANOVA for cultivars representative of clusters.

Source	df	Sum of squares	Mean square	F value	Prob
Cultivars	5	46.833	9.367	2152.6249	**
Chromosomes	12	72.033	6.003	1379.5315	**
Cultivar X chromosome	60	6.076	0.101	23.2724	**
Error	156	0.679	0.004		

C.V= 2.48%

Table 3. Average of genomic characteristics in Iranian *Gossypium herbaceum*.

2n	T.L	L	S	L/S	TF%	C.V	S%	X	D.R.L	T.V	No. SAT
26	35.08	3.89	1.66	2.30	41.17	23.07	43.41	2.65	6.23	7.08	1.88

T.L = Total length, L = longest chromosome, S = shortest chromosome, TF% = total form percentage, C.V. = coefficient of variation, X = mean of chromosome length, D.R.L. = different relative length, T.V. = total volume, No.SAT = number of satellites.

reduced for the length of the long arms (0.72-0.96), length of short arms (-0.01-0.61) and L/S ratio (0.31-0.96), indicating occurrence of structural change of chromosomes, as a result of gene duplication, chromosomal deletion, translocation and heterochromatin change, among the cultivars and pointed towards their distinctness. These rearrangements may have positive effects on adaptability and tolerance to abiotic stress (Gennur et al., 1988).

Determination of the karyotype formulae of different cultivars revealed that almost all the chromosomes were of “m” type (except for 1 or 3 chromosomes which were of “M” type), also indicating presence of homogeneity in chromosome types. Gennur et al. (1988) reported the occurrence of 8.27 metacentric chromosomes, 4.61 submetacentric and 0.11 acrocentric chromosomes in Asiatic cotton *G. herbaceum.* However the present study does not show the occurrence of acrocentric chromosomes in *G. herbaceum* ecotypes available in Iran.

In chromosome analysis, a common practice is to determine the homology of chromosomes in a pair by their length and arm ratio. In order to compare these two methods, Zhou (2003) used the karyotypes of 10 plants species as reference. He measured the length and the size of each pair of chromosomes, checked the results by a χ^2 test and found that the difference between the results obtained by the two methods was insignificant. It was suggested that when the centromere and the length of chromosome is not fully expressed, the method of measuring chromosome size to determine the identity or disparity of chromosomes in some plants is feasible.

When the history of plant evolution is studied, we learn how the genesis of many plants has been established. For example, the basic number of chromosomes of a genome for the genus *Triticum* is 7, that for the genus *Gossypium* is also 7, while those for the genus *Brassica* are 8, 9, 10. From the perspective of basic number, many of the current diploidy plants in fact are homoeologous polyploids.

Islamic Republic of Iran has a diverse climatic condition in different regions. In south of Caspian Sea with mild clime (annual average of Temp. 18^{o}C), West regions with Mediterranean clime, East region with semiarid conditions, and South of Iran with high humidity and high

Figure 2. Dendrogram of 42 *G. herbaceum* varieties generated by karyological data using the UPGMA method (See Table 1 for nomenclature).

temperature (some region with 54°C). All of collected landraces are planted traditionally in around of central areas (falat) of Iran, which have arid, semiarid and desert conditions. According to cluster analysis, the studied cotton varieties divided into four clusters. Most cultivars were presented in the first and second clusters (18 and 13 cultivars, respectively). These results are nearly similar to the morphological characters clustering reported elsewhere (Sheidai and Alishah 1998).

Hybridization program can be arranged with crossing those cultivars placed in distant clusters (differ genetically) to obtain a better combination of parental traits and a high degree of heterosis. For example, members of the cluster 1 can be crossed with the members of cluster 4 to get more variability and heterosis. The variability pattern of local germplasm points to a type of hybrid, which might replace the local variety of a particular region. On the other hand, results of the cluster analysis indicated that some of landraces grown in the same location were placed in different clusters. For example, the Sabzevar landrace (Number 23) was placed in first class, whereas Sabzevar 1 (Number 37) was placed in 3rd class. The Kerman B (number 7) and Kerman G (number 17) located in the same cluster, but Kerman landrace (number 1) was placed in a different class. Therefore, results of this study indicate there is no acceptable relationship between the genomic variation and geographical adaptability. The present study indicates genomic differences among diploid *G. herbaceum* cultivars of Iran, which can be used in the subsequent hybridization programs.

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