Karyological Studies in *Gossypium herbaceum* Cultivars of Iran

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Cotton is one of the most important crops of Iran, cultivated in different regions of the country. Continuous cultivation of improved and uniform cultivars leads to genetic erosion rapidly, hence there is an urgent need to try and assemble whatever diversity still available (Rao and Riley 1994, Fitzgerald 1989).

Gossypium herbaceum is one of the A-genome cottons which is diploid and is potentially important resource for cotton breeding programs (Stanton *et al.* 1994). Though different cultivars of *G. herbaceum* were collected in Iran National Gene Bank from various regions, no information was available on morphology or cytogenetics of these plants until initiation of present study. Present paper considers karyological studies of forty-two *G. herbaceum* cultivars of Iran.

Materials and methods

Forty-two *Gossypium herbaceum* cultivars available in Iran National Gene Bank were planted according to randomized completely block design in Varamin cotton research center during 1994–95. Each cultivar was planted on a line from which 10 plants were selected randomly and used for morphological and cytological studies.

Seeds collected from these plants were germinated in petridish plates after removing the linters and freshly grown roots were used for karyological preparations. The method of pretreatment and fixation followed as before (Sheidai *et al.* 1996a). Different stains were tried and the best result was obtained using 2% acetic-orcein.

Karyotypes were compared using total form percentage (Forni-Martin *et al.* 1994), ratio of the 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.* 1988b) and total volume (Datta and Agarwal 1992).

Karl Pearson coefficient of correlation was determined for total length of chromosomes, length of the long arms, length of the short arms and L/S ratio (Verma 1980), chromosomes were described according to Levan's terminology (Levan *et al.* 1964).

Factorial analysis was performed on karyological data with cultivars and chromosomes as the two factors.

Cluster analysis using UPGMA method (Bernardello *et al.* 1994, Sheidai *et al.* 1996a) and principal coordinate analysis (Pickersgill *et al.* 1976) were used to group karyotypically similar cultivars. NTSYS-pc version 1.4 was used for multivariate statistical analysis. For principal coordinate analysis data were first standardized (mean=0 and variance=1). Taxonomic distance was determined from standardized data, followed by eigen value analysis and ordination of cultivars on the first two principal coordinate axis (Pickersgill *et al.* 1976).

Principal component analysis was performed on the size of chromosomes and chromosome

arms in each karyotype to indicate the most variable chromosome/chromosome arms. For convenience from each cluster a single representative was selected for PCA analysis. For PCA analysis data were first standardized and then product-moment correlation was determined (Sneath and Sokal 1973). Eigen values were determined from transformed data and ordination of cultivars was performed on the first two PCA axis (McClintock and Waterway 1994).

Results and discussion

Names of the cultivars, their karyotype formulae and number of SAT-chromosomes are presented in Table 1. All the cultivars possessed $2n=26$ chromosome number but varied with regard to number of SAT-chromosomes (ranging from 1 to 3) and the chromosomes carrying

Table 1. Names of cultivars, SAT-chromosomes and karyotype formulae.

Cultivar	2n	No. SAT	SAT-Chromosomes	Karyotype formulae
1. Kerman	26	1	1	1 M + 12 m
2. Kashan 12	26	1	1	3 M + 10 m
3. Ghom 1	26	2	1, 3	13 m
4. Giroft	26	2	1, 2	1 M + 12 m
5. Garmsar 60	26	3	1, 2, 3	13 m
6. Lasjard	26	2	1, 2	13 m
7. Kerman B.	26	3	1, 2, 3	13 m
8. Neiriz. R.	26	2	1, 8	13 m
9. Ghom 52	26	2	1, 3	13 m
10. Neiriz GAZ	26	2	1, 2	13 m
11. Shahrood	26	2	2, 4	13 m
12. Damghan	26	2	1, 8	13 m
13. Shahreza	26	2	1, 3	13 m
14. Neishaboor	26	2	1, 4	13 m
15. Ardekan 1	26	2	3, 4	13 m
16. Kashmar	26	2	3, 4	13 m
17. Kerman G.	26	2	5, 7	13 m
18. Hashemabad	26	1	1	1 M + 12 m
19. Esfahan A.	26	2	1, 4	1 M + 12 m
20. Shooshtar	26	2	1, 4	1 M + 12 m
21. Gozagh	26	2	1, 4	1 M + 12 m
22. Mehrizsabz	26	2	2, 4	1 M + 12 m
23. Sabzevar	26	2	1, 3	2 M + 11 m
24. Marvast	26	2	3, 6	13 m
25. Rafsangan	26	3	1, 4, 8	1 M + 12 m
26. Mehrizbah	26	2	1, 5	13 m
27. Ghomgermez	26	1	2	2 M + 11 m
28. Mehrizgod	26	2	1, 3	1 M + 12 m
29. Ardekan 2	26	2	1, 2	13 m
30. Garmsar	26	2	1, 2	13 m
31. Alyafrangi	26	3	1, 2, 6	13 m
32. Bandarabas	26	1	3	13 m
33. Rafsengan 2	26	1	4	1 M + 12 m
34. Rafsengan 3	26	2	1, 2	13 m
35. Harat	26	2	1, 3	13 m
36. Ghom 3	26	2	2, 5	13 m
37. Sabzevar 1	26	2	1, 4	13 m
38. Arya	26	1	1	2 M + 11 m
39. Mehrizgher	26	1	2	3 M + 10 m
40. Mahalat	26	1	3	13 m
41. Mehriz 4	26	2	1, 4	13 m
42. Mehriz 5	26	2	1, 3	2 M + 11 m

secondary constrictions (Fig. 1).

In most of the cases the longest chromosomes of the haploid set possessed secondary constriction but in few cases chromosomes 6, 7 and 8 were SAT-chromosomes (Cultivars 8, 12, 17, 24, 25, and 30, Table 1). The size of SAT also varied from 0.31 μm to 0.78 μm . Almost all

— 9 μm

Fig. 1

Fig. 1. Somatic chromosome numbers (cultivars sequence as Table 1). Figures 1 to 21 are for cultivars 2-4, 6, 8-15, 18-20, 22, 25, 26, 29 and 40 respectively.

the chromosomes were of m type except 1 or 2 chromosomes which were of M type (Table 1). Gennur *et al.* (1988a, b) reported occurrence of 8.27 metacentric chromosomes, 4.61 submetacentric and 0.11 acrocentric chromosomes in Asiatic cotton *G. herbaceum*. However present study does not show occurrence of acrocentric chromosomes in *G. herbaceum* cultivars available in Iran, indicating presence of genomic diversity in this species. As we shall see later on other analysis also support such, assumption.

Details of karyotypes are presented in Table 2. In general karyotypes were symmetrical with small size of chromosomes. The highest total length occurred in Sabzevar cultivar ($46.46 \mu\text{m}$) and the least in Mahalat ($23.83 \mu\text{m}$). The longest chromosome was observed in Sabzevar cultivar ($5.03 \mu\text{m}$) while the shortest was observed in Mahalat ($1.2 \mu\text{m}$). The highest C.V. occurred in Ghom 52 (30.1) indicating more variation among its chromosomes, while the lowest value of C.V. occurred in Mehrizger (13.5).

The mean total length of chromosomes varied from $1.8 \mu\text{m}$ (Mahalat) to $3.5 \mu\text{m}$ (Sabzevar) and the mean total volume varied from 4.7 (Mahalat) to 9.3 (Mehrizbah).

Karl Pearson coefficient of correlation for total length of the chromosomes was >0.95 indicating homogeneity of the group but this value was reduced for the length of long arms (0.72-0.96), length of short arms (-0.01-0.61) and L/S ratio (0.31-0.96) indicating occur-

Table 2. Cultivars and their karyotypic details

Cultivar	T.L	L	S	L/S	TF%	C.V	S%	X	D.R.L	T.V
1. Kerman	31.63	3.53	1.54	2.3	44.0	22.0	51.0	2.3	5.2	6.5
2. Kashan 12	30.65	3.27	1.67	2.0	41.7	20.0	43.6	2.4	6.3	5.8
3. Ghom 1	28.89	3.26	1.33	2.4	40.9	23.0	40.8	2.2	6.7	5.4
4. Giroft	28.31	2.97	1.42	2.1	41.5	19.3	47.0	2.1	5.5	5.1
5. Garmsar 60	46.16	4.99	2.23	2.2	39.7	21.7	42.7	3.5	5.9	8.3
6. Lasjard	34.65	3.75	1.58	2.3	43.3	22.1	42.1	2.6	6.2	6.8
7. Kerman B.	35.23	3.80	1.72	2.2	41.2	22.0	45.2	2.7	5.9	6.5
8. Neiriz R.	36.10	4.31	1.73	2.5	42.5	26.1	40.1	2.7	7.1	6.6
9. Ghom 52	37.44	4.81	1.76	2.7	42.9	30.1	36.4	2.8	8.1	7.0
10. Neiriz GAZ	35.82	4.26	1.58	2.7	38.7	28.5	37.0	2.7	7.4	6.6
11. Shahrood	38.10	4.30	1.66	2.59	40.0	26.0	38.6	2.9	6.9	7.2
12. Damghan	36.17	4.06	1.60	2.4	39.7	23.8	40.0	2.7	6.8	7.2
13. Shahreza	32.03	3.55	1.50	2.3	41.4	23.0	43.7	2.4	6.3	6.7
14. Neishaboor	37.42	4.28	1.67	2.5	39.6	26.0	39.1	2.8	6.9	8.2
15. Ardekan 1	34.68	3.73	1.64	2.2	40.3	22.0	43.0	2.6	6.0	7.0
16. Kashmar	37.20	3.82	1.91	2.0	41.3	19.0	50.0	2.8	5.1	7.9
17. Kerman G.	37.61	3.97	1.82	2.1	41.4	21.8	45.8	2.9	5.7	7.9
18. Hashemabad	31.69	3.57	1.62	2.2	39.4	21.5	45.3	2.4	6.1	6.9
19. Esfahan A.	41.13	4.49	1.76	2.5	41.5	23.7	39.1	3.1	6.6	9.1
20. Shooshtar	36.16	4.05	1.72	2.3	42.1	21.7	42.4	2.8	6.3	8.0
21. Gozagh	35.30	3.66	1.73	2.1	42.1	21.1	47.2	2.7	5.4	7.7
22. Mehrizsabz	30.78	3.45	1.50	2.3	39.8	25.4	43.3	2.3	6.3	6.3
23. Sabzevar	35.74	4.05	1.76	2.3	40.5	26.0	43.5	2.7	6.4	7.4
24. Marvast	30.56	3.09	1.58	1.9	42.3	18.0	51.1	2.3	4.9	6.6
25. Rafsangan	42.66	4.73	1.95	2.4	40.4	25.2	41.2	3.2	6.5	9.0
26. Mehrizbah	42.77	4.96	1.56	3.1	40.6	28.3	31.4	3.3	7.9	9.3
27. Ghomgermez	30.35	3.24	1.60	2.0	40.2	21.5	49.3	2.3	5.4	6.1
28. Mehrizgod	30.08	3.48	1.36	2.5	41.5	25.0	39.0	2.3	7.0	6.1
29. Ardekan 2	38.02	4.08	1.80	2.2	41.6	22.9	44.1	2.9	6.0	8.0
30. Garmsar	32.27	3.40	1.49	2.2	39.4	22.0	43.8	2.4	5.9	6.7
31. Alyafrangi	42.36	4.95	1.82	2.7	40.2	26.0	36.7	3.2	7.8	9.2
32. Bandarabas	38.23	4.48	1.64	2.7	41.5	28.6	36.6	2.9	7.4	7.6
33. Rafsengan 2	34.50	3.90	1.72	2.2	43.1	24.0	44.0	2.6	6.3	6.8
34. Rafsengan 3	35.14	4.13	1.59	2.6	41.7	27.6	38.5	2.7	7.2	7.1
35. Harat	40.82	4.71	1.95	2.4	39.7	24.0	41.0	3.1	6.7	8.2
36. Ghom 3	40.18	4.79	1.82	2.6	39.2	27.0	38.0	3.1	7.4	7.9
37. Sabzevar 1	46.46	5.03	2.01	2.5	41.3	24.8	39.9	3.5	6.5	9.3
38. Arya	27.80	2.71	1.71	1.5	43.2	16.2	63.0	2.1	3.6	5.2
39. Mehrizgher	30.63	2.91	1.75	1.6	43.9	13.5	60.1	2.3	3.7	6.0
40. Mahalat	23.83	2.40	1.20	2.0	40.2	17.3	50.0	1.8	5.0	4.7
41. Mehriz 4	27.41	3.11	1.39	2.2	40.5	20.4	44.6	2.1	6.2	5.7
42. Mehriz 5	30.77	3.46	1.53	2.2	43.2	21.1	44.2	2.3	6.2	6.0

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

rence of structural changes of chromosomes among the cultivars and points towards their distinctness.

ANOVA based on factorial analysis of karyotypic data (Table 3), showed a significant difference among the cultivars.

All these results indicate presence of variation in genetic material of *G. herbaceum* cultivars both quantitatively (size differences) and qualitatively (structural changes), which can be used in hybridization programs to broaden the genetic variability of the gene pool. For this purpose cluster analysis can be used.

Dendrogram produced from UPGMA cluster analysis is presented in Fig. 2. In total

Table 3. ANOVA for cultivars representative of clusters

Source	Degrees of freedom	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 $\times$ Chromosome	60	6.076	0.101	23.2724	**
Error	156	0.679	0.004		
Total	233	125.621			

Coefficient of variation: 2.48%.

Fig. 2. UPGMA cluster analysis (cultivars sequence as Table 1).

cultivars are grouped in six clusters. Nine cultivars are present in each of the first and second cluster, 7 cultivars in the third cluster, 6 in the forth, 8 in the fifth and 3 cultivars in the sixth cluster.

It is suggested to carry out different methods of clustering in order to recognise the real clusters (Ingrouille 1986, Rolf 1987). Other clustering methods such as single linkage and complete linkage was carried out for karyological data and the same results were obtained again indicating distinctness of the clusters (Rohlf 1987). Moreover fit of the clusters obtained to the original data was checked using cophenetic correlation (Stanton *et al.* 1994). Correlation obtained ($r=0.82$) indicates good fit of clusters.

Hybridization program can be arranged with crossing those cultivars placed in distant clusters (differ genetically). In this case members of the cluster 1 can be crossed with cluster 6 to get more variability and heterosis. It is interesting to note that cultivars also varied significantly with regard to many of the morphological characters studied including the yield in the first and second harvest (unpublished data). Presence of variability within *G. herbaceum*

Fig. 3. Plot of cultivars on the first two principal coordinate axis.

has also been noticed by Hutchinson (1950) and partitioned into infraspecific races based on a combination of geographical and morphological factors (Hutchinson 1950).

Grouping of the cultivars was also carried out using principal coordinate analysis. Cultivars were plotted against the first two PCO axis (Fig. 3). Grouping obtained from principal coordinate analysis is very similar to clustering result (Fig. 2) for example cultivars 38, 39 and 40 which form cluster number six are grouped together, cultivars 5, 35, 36, 37 and 25 which belong to cluster number five are grouped together and so on.

Principal component analysis (PCA) was used to indicate the most variable characters (chromosome/chromosome arm) (Chatfield and Collins 1995, Sheidai *et al.* 1996b). Characters with high eigen values can be considered as the most variable one. For this analysis from each cluster (Fig. 2) a single cultivar was selected.

Eigen values obtained from PCA analysis are presented in Table 4. The first two PCA axis comprise 92% of the total variance, hence characters possessing high loadings from these two

Table 4. PCA eigen values (TL=total length, L=long arm and S=short arm)

Character	Eigen values		
1. T.L 1	0.9442355	0.3044422	0.0130737
2. T.L 2	0.9435303	0.3207656	0.0654042
3. T.L 3	0.9616423	0.2595112	-0.0792506
4. T.L 4	0.9802814	0.1661346	-0.0995759
5. T.L 5	0.9842811	0.1730213	-0.0215702
6. T.L 6	0.9761192	0.1459243	0.0623697
7. T.L 7	0.9897339	0.0926357	0.0939711
8. T.L 8	0.9893773	-0.0947087	0.0445466
9. T.L 9	0.9827799	-0.1497337	-0.0078519
10. T.L 10	0.9720023	-0.2260099	-0.0302287
11. T.L 11	0.9557984	-0.2825199	-0.0300870
12. T.L 12	0.9611933	-0.2506495	0.0361333
13. T.L 13	0.9515376	-0.1438370	-0.0871166
14. L 1	0.9296600	0.2994036	0.1796616
15. L 2	0.9792231	0.1452155	0.0024225
16. L 3	0.8932266	0.1047869	0.4004735
17. L 4	0.9517452	0.0494204	-0.0990247
18. L 5	0.7984431	0.5938848	0.0705069
19. L 6	0.8299985	0.5099703	-0.1955794
20. L 7	0.9094835	0.1631144	0.3615534
21. L 8	0.9849548	0.0207943	0.1026338
22. L 9	0.9603475	0.1289418	-0.1504501
23. L 10	0.9822197	-0.0062697	-0.0874987
24. L 11	0.9388434	-0.1774599	0.2415662
25. L 12	0.9552200	-0.2130226	-0.1495943
26. L 13	0.9899347	-0.1180497	-0.0139577
27. S 1	0.6888841	0.3593750	0.3071804
28. S 2	0.8473538	0.4375768	-0.1500808
29. S 3	0.9517440	0.0893563	-0.0368498
30. S 4	0.7864973	0.0694117	-0.5154691
31. S 5	0.7590390	-0.3111688	-0.4373530
32. S 6	0.8489204	-0.3566482	0.3624171
33. S 7	0.9654807	0.0013565	-0.2224166
34. S 8	0.9307960	-0.2570860	-0.0210383
35. S 9	0.8530551	-0.4832312	0.1764230
36. S 10	0.6502419	-0.6928445	0.1186614
37. S 11	0.8172904	-0.3763670	-0.3909865
38. S 12	0.8808297	-0.2938288	0.3629604
39. S 13	0.8665577	-0.1272617	-0.1998353

Fig. 4. Plot of cluster representatives on first two PCA axis (1=cultivar 23, 2=20, 3=22, 4=4, 5=5, 6=39).

axis contribute to the karyotypic variations observed among the cultivars studied.

Result obtained (Table 4) indicates that total chromosome length of chromosomes 4 to 10 with high loading (>0.98), long arms of the chromosomes 2, 8, 10 and 13 (>0.97) and short arms of the chromosomes 3, 7 and 8 (>0.95) are the most variable characters among the cultivars studied. Cophenetic correlation >0.84 indicates good fit of eigen values (Stanton *et al.* 1994).

Ordination of the cultivars selected from each cluster on the first two PCA axis is presented in Fig. 4. Separation of clusters are evident (for example cluster 1 from 6) indicating genomic differences.

Present work reports karyological analysis of the diploid *Gossypium herbaceum* cultivars of Iran for the first time, indicating their genomic differences. Crossing plan with regard to results obtained is in hand.

Summary

Forty-two *G. herbaceum* cultivars available in Iran National Gene Bank were analysed karyologically showing $2n=26$ chromosome number. The cultivars varied with regard to number of SAT-chromosomes and chromosomes carrying secondary constrictions. Karyotypes were of symmetrical type having small chromosomes. Factorial analysis showed significant difference for both the cultivars as well as chromosomes.

Cluster analysis using UPGMA, single linkage and complete linkage methods grouped the cultivars in six distinct clusters. Principal coordinate analysis supports the clustering results and principal component analysis showed that chromosomes 4 to 10 and long arms of the chromosomes 2, 8, 10 and 13 and short arms of the chromosomes 3, 7 and 8 are the most variable chromosomes/chromosome arms among the cultivars studied.

Present work indicates genomic differences among diploid *G. herbaceum* cultivars of Iran which can be used in hybridization programs. Crossing of the cultivars placed in distant clusters are suggested.

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