Karyomorphological Studies in Asiatic Cottons I.

Karyotypic analysis of species and races of Asiatic cottons based on chromatin content

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The species considered for the studies, *Gossypium herbaceum* and *Gossypium arboreum* belong to natural order Malvales, family Malvaceae, subfamily Hibisceae and section Herbacea. *G. herbaceum* has been divided into five races viz., *africanum*, *acerifolium*, *persicum*, *kultjianum*, *wightianum* and *G. arboreum* has been classified into six races viz., *saudanense*, *indicum*, *burmanicum*, *sinense*, *bengalense*, *cernuum*. The karyomorphological studies in Asiatic cottons has been divided into two parts. Part I deals with the chromatin content of the genome, and part II deals with the nucleolar chromosomes and symmetry of the karyotype. Two races of *G. herbaceum* viz., *persicum* and *wightianum* and two races of *G. arboreum* viz., *indicum* and *bengalense* are used in the studies.

Materials and methods

The cotton seeds kindly supplied by the Senior Scientist (Cotton), ARS, Dhawrad were put for germination in the germination box. The actively growing root tips of 0.5 cm were excised at 1.0 PM and pretreated with 0.002 M 8-hydroxyquinoline for 4 hours and were then fixed in acetic alcohol (1:3) for 24 hours. The root tips taken from the fixative were put in 5% pectinase solution for one hour. Then the root tips were hydrolysed in 1N HCl with 1.5% acetocarmine in the proportion of 1:5 drops in a watch glass, by gently heating over the spirit lamp flame till the vapour were seen. The hydrolysed root tips were squashed in 1.5% acetocarmine. The total length of all the chromosomes of a haploid component has been taken as the absolute length. The statistical comparisons were made by applying 't' test. In the idiograms the secondary constrictions are differentiated from satellites by leaving blank in the secondary constriction chromosomes.

Results and discussion

The karyotypic analysis of *G. herbaceum*, *G. arboreum* and their races was made on the basis of largest chromosome length, smallest chromosome length and absolute length. The smallest chromosome length of *G. herbaceum* and *G. arboreum* was 1.713 μ and 1.322 μ respectively. The race *wightianum* had shorter smallest chromosome length (1.408 μ) compared to race *persicum* (1.830 μ) in *G. herbaceum* (Table 1) while *G. arboreum* race *bengalense* had shorter smallest chromosome length (1.269 μ) compared to race *indicum* (1.385 μ) (Table 2).

The largest chromosome length of *G. herbaceum* and *G. arboreum* was 2.814 μ and 2.319 μ respectively. The largest chromosome length of race *persicum* (2.914 μ) was greater than that of the race *wightianum* (2.555 μ) in *G. herbaceum* (Table 1). In *G. arboreum* the largest chromosome length of race *indicum* (2.402 μ) was greater than that of the race *bengalense* (2.248 μ)

(Table 2).

Absolute chromosome length of *G. herbaceum* was 29.547 μ and that of *G. arboreum* was 23.827 μ . In case of races *persicum* and *wightianum* of *G. herbaceum* it was 30.516 μ and 27.025 μ respectively while in case of *indicum* and *bengalense* of *G. arboreum* it was 25.060 μ and 22.770 μ respectively (Tables 1, 2).

From the statistical analysis it was clear that the reduction in absolute chromosome length from *G. herbaceum* to *G. arboreum* at species level and from *persicum* to *wightianum* and *indicum* to *bengalense* at racial level was significant (Tables 1, 2, 3).

Table 1. Racial differences of chromosome length in *Gossypium herbaceum*

Rece/Culture <i>G. herbaceum</i>	Smallest chromosome (μ)	Largest chromosome (μ)	Absolute length (μ)
Race <i>persicum</i>			
1449	1.890	3.265	34.401
5497	1.875	3.250	34.310
6455	1.869	3.243	34.196
DM 125	1.945	2.830	30.684
SM 14	2.000	2.687	30.516
SM 150	1.937	2.812	30.371
SM 43	1.930	2.890	30.327
SM 81	1.725	2.940	29.152
SM 41	1.625	2.937	28.997
SM 141	1.700	2.824	28.686
SM 73	1.800	2.705	28.662
SM 143	1.750	2.750	28.289
SM 88	1.750	2.750	28.123
Range	1.625–2.000	2.687–3.265	28.123–34.401
Average of race	1.830	2.914	30.516*
Variance (σ^2)	—	—	5.396
S. Em. $\pm$	—	—	0.644
Race <i>wightianum</i>			
Jayadhar	1.187	2.562	27.506
R 51–238	1.480	2.715	27.365
MDS 42	1.499	2.500	26.936
DB 3–12	1.500	2.625	26.810
2 5-B-6	1.375	2.375	26.509
Range	1.187–1.500	2.375–2.715	26.509–27.506
Average of race	1.408	2.555	27.025
Variance (σ^2)	—	—	0.166
S. Em. $\pm$	—	—	0.182
Calculated t	—	—	5.123
Table t	—	—	2.220
Average of species	1.713	2.814	29.547

* *persicum* significantly differs from *wightianum* at 5% level.

Mikhailova (1936) did not notice any appreciable difference in the size of chromosome and concluded that the karyotypes of Asiatic cottons were substantially the same but on the contrary in the present investigation lot of variation was observed (Plate 1). With regard to absolute length, the present findings agreed with the findings of Tutajuk and Sadytlova (1967) that the somatic chromosomes of *G. herbaceum* were relatively larger compared to *G. arboreum* but the statement of Skovsted (1933) that the somatic chromosomes of *G. arboreum* were of equal sizes did not hold true since there was lot of variation. The present investigation did not

support the analysis of Jacob (1941, 1942) that the shortest chromosomes of all the *herbaceum* types (races) were nearly equal since there was variation amongst the races of *G. herbaceum* with respect to shortest chromosomes (Plate 1).

The genetic and evolutionary significance of heterochromatin has been reported by Darlington and Mather (1950). They pointed out that in some cases the loss of heterochromatin

Table 2. Racial differences of chromosome length in *Gossypium arboreum*

Race/Culture <i>G. arboreum</i>	Smallest chromosome (μ)	Largest chromosome (μ)	Absolute length (μ)
<i>Race indicum</i>			
1187	1.562	2.325	25.779
853	1.250	2.499	25.621
860	1.437	2.437	25.184
1042	1.562	2.525	24.834
824	1.437	2.375	24.821
875	1.062	2.250	24.122
Range	1.062–1.562	2.250–2.525	24.122–25.779
Average of race	1.385	2.402	25.060*
Variance (σ^2)	—	—	0.367
S. Em. $\pm$	—	—	0.247
<i>race bengalense</i>			
LD 124	1.312	2.250	25.185
LD 132	1.500	2.312	23.185
Lohit	1.337	2.350	23.123
G 27	1.150	2.412	22.810
LD 133	1.087	2.162	22.372
LD 135	1.250	2.125	21.373
HD 11	1.250	2.125	21.340
Range	1.087–1.500	1.125–2.412	21.340–25.185
Average of race	1.269	2.248	22.770
Variance (σ^2)	—	—	1.714
S. Em $\pm$	—	—	0.495
Calculated t	—	—	3.930
Table t	—	—	2.201
Average of species	1.322	2.319	23.827

* *indicum* significantly differs from *bengalense* at 5% level.

Table 3. Comparison of *G. herbaceum* and *G. arboreum* with respect to absolute length

	<i>G. herbaceum</i>	<i>G. arboreum</i>
Average absolute length (μ)	29.547*	23.827
Variance	6.079	2.236
S. Em $\pm$	0.581	0.414
Calculated t	7.486	—
Table t	1.119	—

* *herbaceum* significantly differs from *arboreum* at 5% level.

segments could be tolerated by the species and it rather adds to their selection value. Sen and Tiwari (1966) noticed changes in the chromosome size and subsequent alterations in the karyotypes of cultures of *Pisum sativum* which was due to loss of heterochromatic material. Mursal and Endrizzi (1976) observed increased 'A' genome size compared to 'D' genome size in *G. hirsutum* due to the presence of more repetitive DNA in 'A' genome. Edwards (1977)

noticed that absolute length of *G. herbaceum* race *africanum* which is the wild form of Asiatic cottons, was 33.06μ . In the present study absolute length of races *persicum* and *wightianum* of *G. herbaceum* was 30.516μ and 27.025μ respectively. Hence, it is an indication of the reduction in chromosome size from race *africanum* to *persicum* and *wightianum* due to removal of repetitive DNA in the course of evolution.

Plate 1. Figs. 1-31. Idiograms of cultures of races *persicum* (1-13), *wightianum* (14-18), *indicum* (19-24) and *bengalense* (25-31). 1, 1449; 2, 5497; 3, 6455; 4, DM 125; 5, SM 14; 6, SM 150; 7, SM 43; 8, SM 81; 9, SM 41; 10, SM 141; 11, SM 73; 12, SM 143; 13, SM 88; 14, Jayadhar; 15, R 51-238; 16, MDS 42; 17, DB 3-12; 18, 2 5-B-6; 19, 1187; 20, 853; 21, 860; 22, 1042; 23, 824; 24, 875; 25, LD 124; 26, LD 132; 27, Lohit; 28, G 27; 29, LD 133; 30, LD 135; 31, HD 11.

In the study of largest, smallest and absolute length it was noticed that there was reduction in the size of the chromosome from species to species and race to race (Tables 1, 2). Differences in absolute chromosome size reflects different amounts of gene duplication either in tandem fashion or through polytene multiplication of chromonemata and also that the species having greater chromatin length were supposed to be primitive where as species with lesser chromatin

length were treated as advanced (Stebbins 1971). Therefore, amongst the two species, *G. herbaceum* is primitive compared to *G. arboreum*. Among two races of *G. herbaceum*, *persicum*

Plate 2. Figs. 1-9. Metaphase plates of cultures of races *persicum* (1, 5497; 2, SM 88), *wightianum* (3, 2 5-B-6), *indicum* (4, 860; 5, 1042; 6, 824; 7, 875.) and flowers (8) and bolls (9) of all races.

is relatively primitive compared to *wightianum* and among two races of *G. arboreum*, *indicum* is primitive compared to *bengalense*.

Plate 3. Figs. 10-13. Bracteoles, petals, lint and leaves of races *persicum*, *wightianum*, *indicum* and *bengalense*. Figs. 14-17. Plants of races *persicum*, *wightianum*, *indicum* and *bengalense*.

Table 4. Racial differences of qualitative characters in *Gossypium herbaceum* and *Gossypium arboreum*

Characters	<i>Gossypium herbaceum</i>			<i>Gossypium arboreum</i>	
	race <i>persicum</i>	race <i>wightianum</i>	race <i>indicum</i>	race <i>bengalense</i>	
Colour of flower	Yellow	Yellow	Yellow	White, purpl, yellow	
Bracteole	Flaring widely from flower and capsule. Rounded or broadly triangular, broader than long, cordate. Margin serrated into 6-8 broadly triangular teeth.	Flaring widely from flower and capsule. Rounded or broadly triangular, broader than long, cordate. Margin serrated into 6-8 broadly triangular teeth.	Closely attached to bud, flower and capsule. More or less triangular, longer than broad, with 3-4 coarse teeth.	Closely attached to bud, flower and capsule. More or less triangular, longer than broad, entire.	
Boll shape	Round	Round	Round	Tapering	
Number of loculi per boll	3	3	Usually 3, rarely 4	Usually 3, very rarely 4	
Opening of boll	Incomplete-complete	Incomplete-complete	Incomplete-complete	Complete	
Colour of the lint	White	White	White	Dull white-white	
Leaf lobe	Ovate to rounded, broad, less than 1/2 cut.	Ovate to rounded, broad, 2/3 cut.	Ovate, oblong, broad, 1/2-2/3 cut.	Lanceolate, narrow, acute, 2/3-4/5 cut.	
Number of lobes per leaf.	Usually 5, rarely 6	Usually 5, rarely 6	Usually 5, rarely 6	Usually 5, rarely 6 or 7	
Colour of leaf	Light green	Light green	Light green	Dark green	
Incision of leaf blade	Palmatifid	Palmatifid	Palmatifid	Palmatipartite	
Stem colour	Green	Green	Green	Red, green	

From the present karyotypic analysis it has been concluded that *G. arboreum* has evolved as a result of loss of redundant DNA from *G. herbaceum* in the course of evolution in addition to the major change of one translocation as indicated by Gerstel (1953) which has been confirmed by Gennur *et al.* (1986).

The present findings are in line with the earlier findings of Babcock and Cameron (1934) and Stebbins (1950) in different plant species that there was change in plant habit as there was reduction in absolute chromosome size, since *G. herbaceum* race *africanum* with perennial habit had 33.06μ absolute length (Edwards 1977) where as races *persicum* and *wightianum* had 30.516μ and 27.025μ respectively with annual habits.

Though there was phylogentic reduction in the absolute chromosome size from *persicum* to *wightianum* in *G. herbaceum* there was no difference between both the races with respect to qualitative characters (Plate 2, Figs. 8, 9; Plate 3) (Table 3). This is similar to the case of *Taraxacum* wherein the reduction in absolute chromosome size has not always been accompanied by the morphological specialisation like decrease in the size of floral parts or change in the colour of leaves or any other plant part (Stebbins 1950). Reduction in absolute chromosome size was also observed in the race of *G. arboreum*, since *indicum* had absolute chromosome size of 25.060μ and *bengalense* had 22.770μ . In *G. arboreum* the reduction in absolute chromosome size of races was associated with drastic morphological variations in the plant parts with regard to qualitative characters. The cultures of *indicum* had light green palmatifid leaves and yellow flowers where as cultures of *bengalense* had dark green palmatipartite leaves, smaller flowers with different colours like yellow, pink and white. Apart from it there were many acute differences between *indicum* and *bengalense* plant habits (Plate 2, Figs. 8, 9; Plate 3) (Table 3).

Summary

Karyotypic analysis of *Gossypium herbaceum* (races *persicum* and *wightianum*) and *G. arboreum* (race *indicum* and *bengalense*) with two races in each species has been made on the basis of smallest, largest and absolute chromosome length. It is concluded that at species level *G. herbaceum* is primitive to *G. arobreum* and at racial level *persicum* is primitive to *wightianum* in *G. herbaceum* and *indicum* is primitive to *bengalense* in *G. arboreum*.

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