APPENDIX

ESTIMATION OF DNA AND PROTEIN IN THE TAXA INVESTIGATED

A number of biochemical and cytochemical studies have been made on deoxyribonucleic acid (DNA) in various plant species (Sato, 1963; Rothfels et al., 1966; EL-Lakany, 1972; SZ-Borsos, 1973; and Nagl, et al., 1973). A correlation of DNA-content with ploidy levels and total chromatin matter made, suggested that DNA-content per nucleus of a somatic cell is constant in each species (Sato, 1963). This supports the so-called "DNA-constant" hypothesis. Thus there is some relationship between the chromosome number and DNA-content per nucleus. On the other hand, some exceptional cases in which this relation is not recognized, have been reported in animal and plant materials (Evans, 1956; Pasteels and Lison, 1951). Nagl et al. (1973) while working with the family Asteraceae have shown the phylogenetic reduction in chromosome size is closely correlated with a reduced DNA-content of diploid nuclei.

Most of these studies have been made on cultivated taxa, while very little is known about the wild taxa. Therefore, in the present study, it was thought that

it would be of interest to see whether a similar type of correlation is prevailing in the taxa studied in the present work.

The study includes the estimation of DNA and protein of 17 species belonging to different genera of the family Malvaceae. Protein was estimated to see the ratio of increase or decrease in protein-content with the increase or decrease in DNA-content.

Materials and Methods :-

Seedlings of open cotyledon stage (7 days old) were taken for the estimation. The estimation of DNA and protein was done for the taxa listed in Table 1.

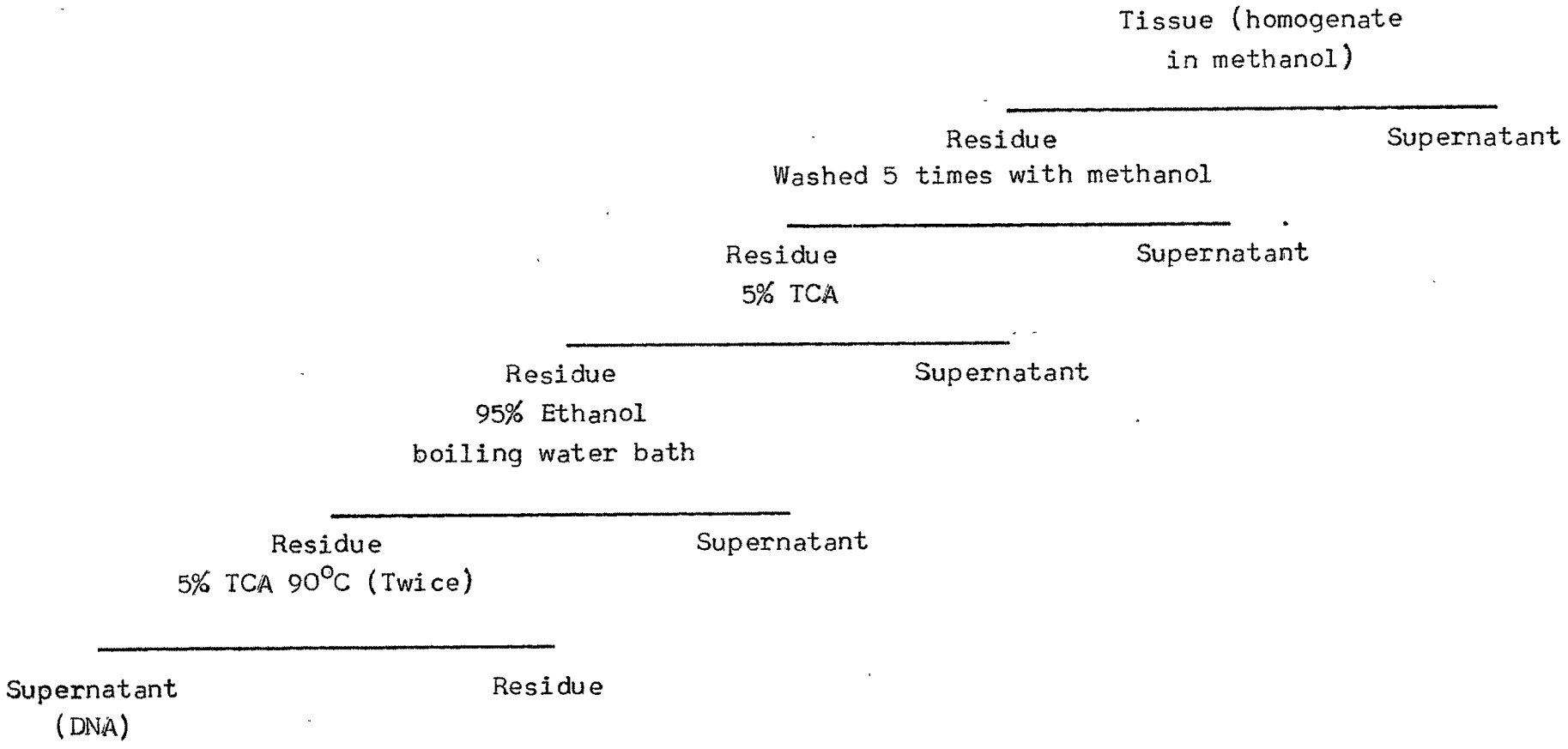
Preparation of extract :-

A 20% homogenate of the radical free seedlings was prepared by grinding in pestle and morter for 20 min. in methanol at room temperature. This homogenate was used for the estimation of DNA by the method used by Schneider (1957) and protein by that of Lowry et al.(1951).

Estimation of DNA :-

The determination of DNA is based on its preferential solubility in hot trichloroacetic acid, which is quantitated by means of colorimetric reactions involving the pentose component of the same.

5 ml of homogenate was centrifuged at 1500 x g for 10 min. and the residue obtained was made pigment free by giving 5 to 6 washes in methanol, following the method of Smillie and Krotkov (1960). This residue was suspended in 2.5 ml of 5% TCA and allowed to stand for 15 min. at room temperature. This was then centrifuged for 15 min. at 1500 x g. The residue obtained was suspended in 5 ml of 95% ethanol, kept in boiling water bath for 30 seconds, cooled and centrifuged at 1500 x g for 15 min. and suspended in 2 ml of 5% TCA, which was heated at 90°C for 15 min. by occasional stirring. This was cooled and centrifuged at 4,000 x g for 15 min.. The supernatent was collected. The residue was washed with 2 ml of 5% TCA and supernatent obtained was added to the previous collection. This was used for the estimation of DNA using Diphenylamine reagent and the blue colour developed was read at 660 nm. using Carl Zeiss spectrophotometer. Purified calf thymus DNA was used as standard (Fig. 1).


Tissue (homogenate
in methanol)
Residue
Supernatant
Washed 5 times with methanol
Residue
Supernatant
5% TCA
Residue
Supernatant
95% Ethanol
boiling water bath
Residue
Supernatant
5% TCA 90°C (Twice)
Supernatant
(DNA)
Residue

Estimation of Protein :-

0.2 ml of homogenate was centrifuged after adding 2 ml of methanol and the residue was made pigment free as mentioned in the case of DNA. The residue obtained was suspended in 1 ml of 10% TCA for 15 min. at room temperature and centrifuged at 1500 x g. The residue was dissolved in 10 ml of 0.1 N NaOH and protein was estimated by the method of Lowry, et al. (1951). Bovine albumine was used as standard

Results and Discussion :-

As mentioned earlier, the DNA and protein content of seedlings of various plant species belonging to the family, Malvaceae are estimated and summarized in Table I. As can be seen from the table that the DNA concentration ranges from 0.05% to 0.14% dry weight, where as the protein concentration ranges from 4.2% to 9.2% of dry weight. It has been noticed from DNA-protein ratio that there is a correlation between the DNA and protein contents except in Thespesia populnea.

TABLE - I

Comparison of the DNA and Protein contents in seedlings of different taxa.

Sr. No.	Name of the taxa	DNA		Protein		DNA/ protein x 100
		mg/g fresh wt.	g/100 g dry wt.	mg/g fresh wt.	g/100 g dry wt.	
1.	Thespesia populnea	0.57	0.14	18.7	4.7	3.05
2.	Azanza lampas	0.43	0.10	35.0	8.3	1.21
3.	Hibiscus panduraeformis	0.44	0.11	24.0	5.9	1.83
4.	H. ovalifolius	0.29	0.06	19.0	4.2	1.52
5.	H. cannabinus	0.27	0.07	19.3	4.7	1.40
6.	H. caesius	0.29	0.07	24.6	5.7	1.17
7.	H. vitifolius	0.36	0.09	21.9	5.6	1.64
8.	H. hirtus	0.44	0.10	26.3	6.3	1.66
9.	H. lobatus	0.25	0.06	18.5	4.6	1.35
10.	H. sabdariffa	0.30	0.07	19.5	4.9	1.54
11.	H. mutabilis	0.31	0.08	22.5	5.6	1.37
12.	Abelmoschus angulosus	0.38	0.09	33.7	8.4	1.11
13.	A. manihot	0.20	0.05	24.4	6.2	0.82
14.	Pavonia patens	0.36	0.08	31.9	7.1	1.11
15.	P.zeylanica	0.39	0.09	39.4	9.2	0.98
16.	Urena lobata	0.28	0.07	25.8	6.4	1.10
17.	Malachra capitata	0.31	0.07	18.1	4.2	1.71

(Mean of 3 to 4 trials).

TABLE - II

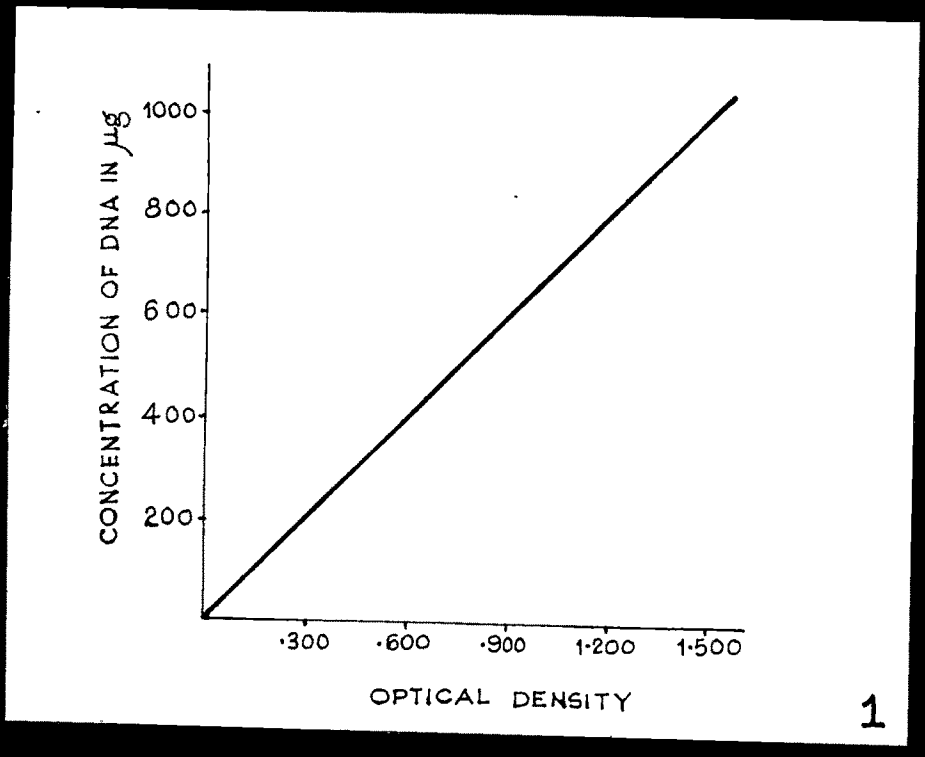
Comparison of total chromatin length with DNA-content of the taxa investigated.

Sr. No.	Taxa	Coll. No.	2 n	Total chromatin length in	DNA mg/g fresh wt.
1.	Thespesia populnea	49	28	121.04	0.57
2.	Azanza lampas	31	28	59.47	0.43
3.	Hibiscus panduraeformis	26	24	82.79	0.44
4.	H. ovalifolius	56	32	69.36	0.29
5.	H. cannabinus	14	36	89.42	0.27
6.	H. caesius	55	36	86.58	0.29
7.	H. vitifolius	33	34	114.70	0.36
8.	H. hirtus	54	64	98.70	0.44
9.	H.lobatus	15	72	72.15	0.25
10.	H. sabdariffa	7	72	110.45	0.30
11.	H. mutabilis	44	120	180.57	0.31
12.	Abelmoschus angulosus	52	130	207.13	0.38
13.	A. manihot	8	130	147.63	0.20
14.	Pavonia patens	16	28	63.58	0.36
15.	P. zeylanica	40	56	143.31	0.39
16.	Urena lobata	29	28	61.71	0.28
17.	Malachra capitata	36	56	76.64	0.31

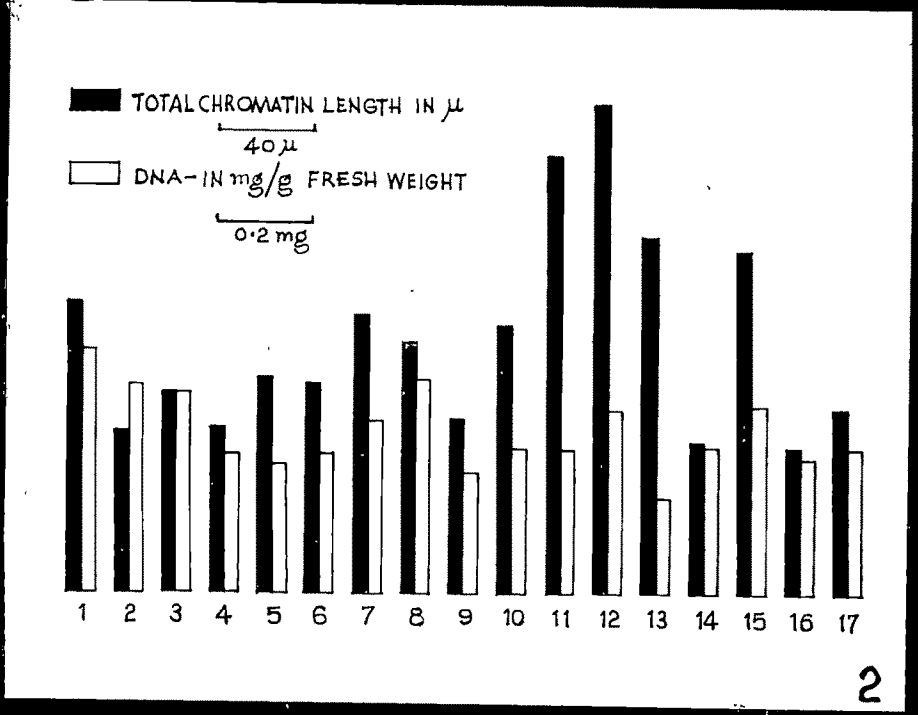
Fig. 1 - Standard DNA graph.

Fig. 2 - Comparison of total chromatin length with DNA-concentration in :

1. *Thespesia populnea*
2. *Azanza lampas*
3. *Hibiscus panduraeformis*
4. *H. ovalifolius*
5. *H. cannabinus*
6. *H. caesius*
7. *H. vitifolius*
8. *H. hirtus*
9. *H. lobatus*
10. *H. sabdariffa*
11. *H. mutabilis*
12. *Abelmoschus angulosus*
13. *A. manihot*
14. *Pavonia patens*
15. *P. zeylanica*
16. *Urena lobata*
17. *Malachra capitata*


CONCENTRATION OF DNA IN μg
1000
800
600
400
200
·300
·600
·900
1·200
1·500
OPTICAL DENSITY

1


TOTAL CHROMATIN LENGTH IN μ
40 μ
DNA-IN mg/g FRESH WEIGHT
0·2 mg
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

2

Comparison has been made, of the total chromatin length and the DNA content (Table II, Fig. 2) to see whether any correlation is existing between the two. It is noticed that the taxa with more chromatin length has higher DNA-content. However, in a few cases such as Abelmoschus manihot, Abelmoschus angulosus and Hibiscus mutabilis although the total chromatin length is high, a comparative increase in DNA-content was not noticed.

REFERENCES

EL-LAKANY, M. H. 1972 Quantitative variation in DNA as related to ploidy level and species in some roses. Can. J. Genet. Cytol. 14(2) : 347-351.

EVANS, W. L. 1956 The effect of cold treatment on the deoxyribonucleic acid (DNA). Cytologia, 21 : 417-432.

LOWRY, O. H.; ROSENBROUGH, N. J. ; FARR, A. L. AND RANDALL, R. J. 1951 Protein measurement with the Folin-Phenol reagent. J. Biol. Chem. 193 : 266-275.

NAGL; WALTER AND FRIEDRICH EHRENDORFER 1973 Chromosome size and DNA-content in three species of Asteraceae - Anthemideae. Oesterr Bot. Z. 121(3/4) : 165-169.

PASTEELES, J. AND L. LISON 1951 Deoxyribonucleic acid content of egg of Sabellaria during maturation and fertilization. Nature 167 : 948-949.

ROTHFELS, K.; SEXSMITH, E.; HEIMBURGER, M. AND KRANS, M. O. 1966 Chromosome size and DNA content of species of Anemone L. and related genera (Ranunculaceae). Chromosoma (Berl.) 20 : 54-74.

SATO, S. I. 1963 Cytophotometrical study of DNA amount in polyploid species of Tradescantia. Sci. Rep. Fac. Lit. Sci. Hirosaki Univ. 10(2) : 73-78.

SCHNEIDER, W. C. 1957 Determination of nucleic acid in tissue by pentose analysis. In "Methods in Enzymology" (Eds.) S. P. Colowick and O. N. Kaplan ed. III : 680-681. Academic Press. New York.

SMILLIE, R. M. AND KROTKOV, G. 1960 The estimation of nucleic acid in some algae and higher plants. Can. J. Bot. 38 : 30-49.

SZ-BORSOS, OLGA 1973 Cytophotometric studies on the DNA-contents of diploid Lotus species. Acta. Bot. Acad. Sci. Hung. 18(1/2) : 49-58.

Cytologia 41: 207–217, 1976

Cytotaxonomy of Malvaceae
I. Chromosome number and karyotype analysis of *Hibiscus, Azanza* and *Urena*

R. P. Bhatt and Aparna Dasgupta

Department of Botany, Faculty of Science, M. S. University of Baroda, Baroda, India

Received August 30, 1974

The Malvaceae include 700 species distributed among 57 genera, chiefly confined to warm temperate regions of the world. Genera *Hibiscus* and *Azanza*, selected for the present investigation belong to the tribe Hibisceae while the genus *Urena* belongs to the tribe Ureneae.

Genus *Hibiscus* is represented by 32 species in India, of which five species viz. *H. vitifolius, H. sabdariffa, H. cannabinus, H. lobatus, H. panduraeformis* occurring in this part of Gujarat, have been dealt with. Many workers have studied this genus for cytological or cytogenetical investigations. Still no definite conclusions are arrived at regarding the basic number, inter-relationships and evolutionary trends within the genus. An attempt is made in the present work to understand the course of evolution in the light of our findings.

The position of *Azanza lampas* has always been problematic. This taxon was placed in the genus *Thespesia* and later transferred to *Hibiscus*, Of late, taxonomists have favoured a separate generic status for this plant (Raizada 1966). Kundu and Rakshit (1970) in their revision of the Indian species of *Hibiscus*, based on morphological characters, suggest that this species may represent a link between the two genera *Hibiscus* and *Thespesia*. As no record of the cytological work is available, this was selected for karyomorphological study to understand its status more correctly.

Urena sinuata has been earlier studied by Skovsted (1941); Hazra and Sharma (1971). The reinvestigation of the species was undertaken with a view to ascertain the existence of cytotypes within the circumscription of species as delimited by taxonomists.

Materials and methods

Excised root tips were pretreated with different chemicals, of which saturated solution of para-dichloro-benzene gave the best results. The treatment was administered for 1.5 to 2 hrs at 10°–12°C. Treated root tips were fixed in acetic-alcohol mixture (1 : 1) for at least one hour before squashing following Tjio and Levan's (1950) aceto-orcein squash method.

Flower buds of the desired size were collected from the plants grown in the University botanical garden. Buds were fixed in acetic-alcohol mixture (1 : 3) between 1.30 to 2.30 p. m. Before, smear preparations in 2% acetocarmine, buds were transferred to 45% acetic-acid for improving staining.

Observations

The following categorization of chromosomes has been made, with a view to describe the karyotype and to represent the same by karyotype formulae. This facilitates the understanding of intraspecific and intergeneric relationships of the taxa analysed.

Type

A— Long chromosome (more than 4 μ) with median or nearly median centromere
B— Long chromosome (more than 4 μ) with sub-median centromere.
C— Long chromosome (more than 4 μ) with sub-terminal centromere.
D— Medium chromosome (2 to 4 μ) with median or nearly median centromere.
E— Medium chromosome (2 to 4 μ) with sub-median centromere.
F— Medium chromosome (2 to 4 μ) with sub-terminal centromere.
G— Short chromosome (1 to 2 μ) with median or nearly median centromere.
H— Short chromosome (1 to 2 μ) with sub-median centromere.
I— Short chromosome (1 to 2 μ) with sub-terminal centromere.
J— Very short chromosome (less than 1 μ) with median or nearly median centromere.
K— Very short chromosome (less than 1 μ) with sub-median centromere.
L— Very short chromosome (less than 1 μ) with subterminal centromere.

Superscript

s— denotes the presence of satellite
s'— denotes the presence of secondary constriction on short arm.
'— denotes the presence of secondary constriction on long arm.

Hibiscus

1) *Hibiscus vitifolius* Lin.:
Absolute length—114.5 μ; Range—4.619 μ to 2.31 μ
$2n=34=B_4+B_2'+D_2+E_{18}+E'_6+E_2^{s'}$

The present report 2n=34 confirms the earlier reports for the species (Skovsted 1935, 1941, Hazra and Sharma 1971). The detailed karyotype analysis reveals the presence of uniformly graded, long and medium types of chromosomes. Majority of chromosomes in the complement is with sub-median centromeres. Out of the five pairs of chromosomes with secondary constriction, four pairs have secondary constriction on long arm, while the fifth pair (E-type) is having secondary constriction on the short arm (Fig. 1 and 1-A). Hazra and Sharma (1971) have not mentioned the occurrence of secondary constriction on short arm in the taxon they have investigated. Skovsted (1935 and 1941) has reported the presence of one pair of satellited chromosome and β-chromosome. In the present work as well as in the work of Hazra and Sharma (1971) these have not been observed.

2) *H. sabdariffa* Linn.:
Absolute length—110.4 μ; Range—2.252 μ to 0.782 μ

$2n=72=D_2+E_6+G_4+H_{44}+H_4^s+I_6+K_6$

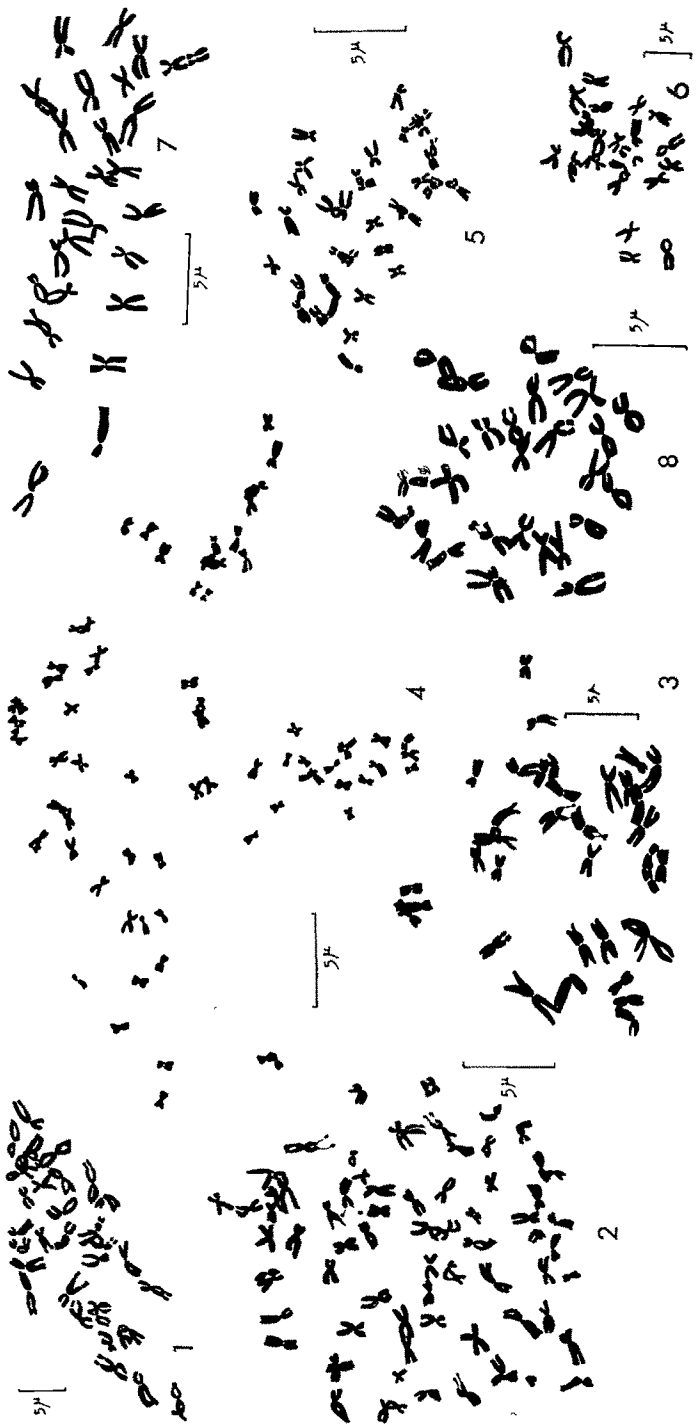
Earlier reports of 2n=36 and 72 are confirmed. The somatic tissue showing diploid and tetraploid segments, was occassionally noticed. Both segments scrutinized for karyomorphological study, showed great similarity in the types of chromosomes in the complement. This indicates that the tetraploid tissue must have arisen in an autotetraploid manner. The chromosomes reveal the greater size difference,

Table 1. Chromosome numbers of the species investigated

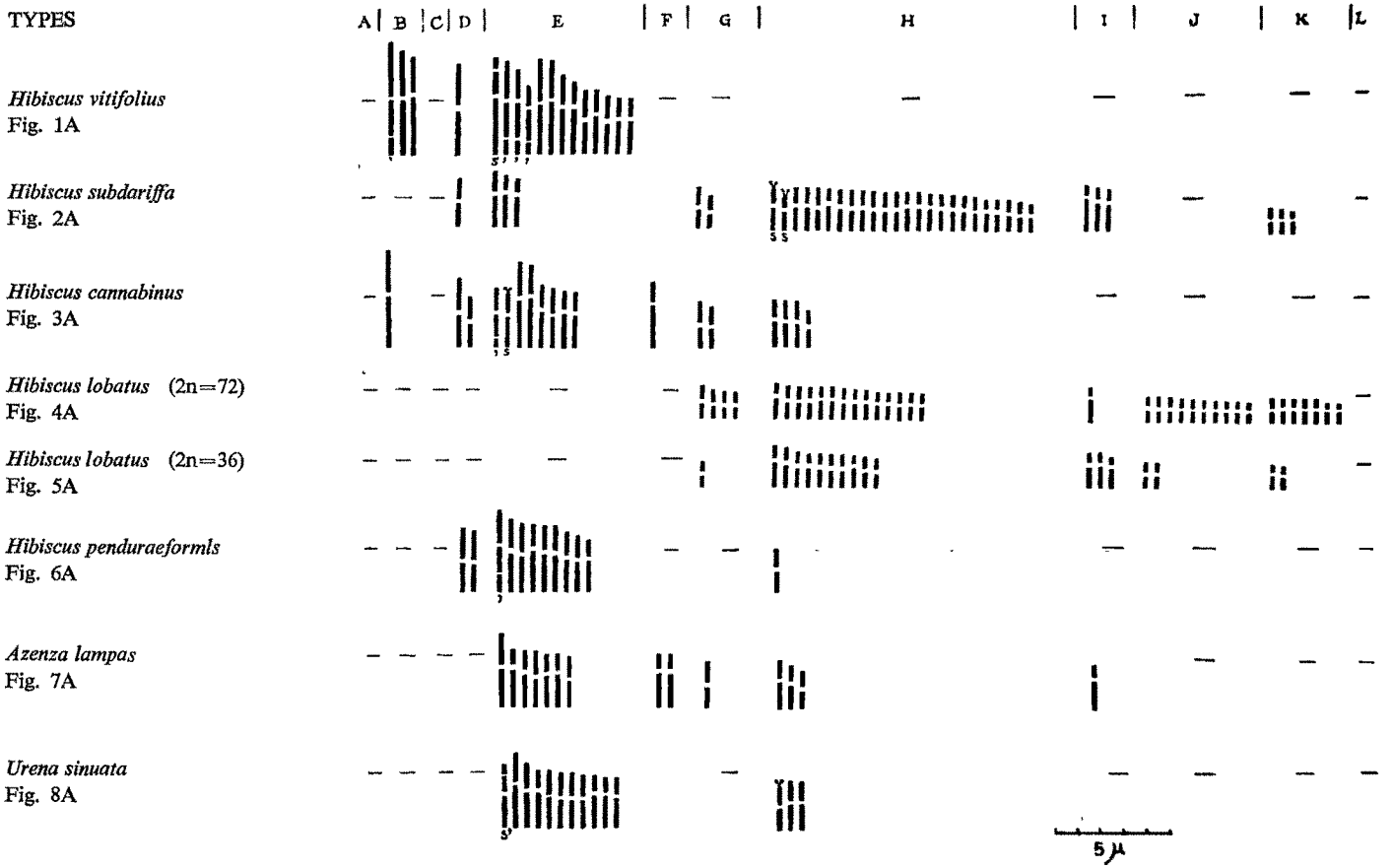
	Name of the species	Previous reports		Present reports
		Authors	*Numbers*	
1.	*H. vitifolius* Linn. Coll. no. 33	Skovsted (1941)	2n=34+0 to 1	2n=34
		Hazra and Sharma (1971)	2n=34	
2.	*H. sabdariffa* Linn. Coll. no. 7	Rao (1935)	2n=72	2n=36,72
		Skovsted (1941)	2n=36, 72	n=36
		Tjio (1948)	2n=72	
		Menzel and Wilson (1963)	n=36	
		Chennaveeraiah and Subbarao (1963)	2n=72	
		Kachecheba (1972)	2n=36	
3.	*H. cannabinus* Linn. Coll. no. 14	Rao (1935)	2n=72	2n=36
		Medvedeva (1936)	2n=36	
		Skovsted (1941)	2n=36	
		Tjio (1948)	2n=36	
		Henzel and Wilson (1961)	n=18	
		Chennaveeraiah and Subbarao (1965)	2n=36	
4.	*H. lobatus* (Murr) Kuntze =*H. solandra* L. Herr Coll. nos. 5 and 15	Medvedeva (1936)	2n=34	2n=36, 72
		Skovsted (1935)	2n=34, 68 n=17	n=18, 36
5.	*H. pandurasformis* Coll. no. 3	Skovsted (1941)	2n=24	2n=24 n=12
6.	*Azanza lampas* (Cav.) Alef. Coll. no. 12	——	——	2n=28
7.	*Urena sinuata* Linn. Coll. no. 29	Skovsted (1941)	2n=28	
		Hazra and Sharma (1971)	2n=28 n=14	2n=28

ranging from long to very short types (Figs. 2 and 2–A). Skovsted (1941) observed one pair of satellited chromosome in a tetraploid taxon, Chennaveeraiah and Subbarao (1965) have reported 7 and 8 pairs of SATs in the two strains studied, Kachecheba (1972) reported 3 pairs of SATs in a diploid taxon, while the present investigation reveals the presence of two pairs of satellited chromosomes in a tetraploid tissue and one pair in a diploid tissue.

3) *H. Cannabinus* Linn.:
Absolute length —89.2 μ; Range-4.2 μ to 1.51 μ.



Figs. 1–8. Somatic metaphase plates of *H. vitifolius*; *H. sabdariffa*; *H. cannabinus*; *H. lobatus* (2n=72); *H. lobatus* (2n=36); *H. pandurae formis*; *Azanza lampas* and *Urena sinuata* respectively.



Figs. 1A–8A. Chromosome types in *H. vitifolius*; *H. sabdariffa*; *H. cannabinus*; *H. lobatus* (2n=72); *H. lobatus* (2n=36); *H. panduraeformis*; *Azanza lampas* and *Urena sinuata* respectively.

$2n=36=B_2+D_4+E_{12}+E_2'+E_2^s+F_2+G_4+H_8$

The present report of 2n=36 is in agreement with those of all the earlier workers except that of Rao (1935). 4 different strains of *H. cannabinus* studied by Chennaveeraiah and Subbarao (1965) differ karyomorphologically with respect to number of SATs and nature of primary constrictions. The population studied here showed chromosomes of uniformly graded nature from long to short type. Majority of the chromosomes are with sub-median centromeres. In addition, to Skovsted's observation of one pair of satellited chromosomes the presence of one pair of chromosomes (E type) with secondary constriction on long arm was noticed in the present work (Figs. 3 and 3-A).

4) *H. lobatus* (Murr.) Kuntze:
=*H. solandra* L. Herr.

Coll. no. 15. Absolute length—72.14 μ; Range-1.36 μ to 0.68 μ.
$2n=72=G_8+H_{28}+I_2+J_{20}+K_{14}$

Coll. no. 5. Absolute length —44.18 μ; Range-1.7 μ to 0.85 μ.
$2n=36=G_2+H_{20}+I_6+J_4+K_4$

Two populations (Coll. no. 5 and Coll. no. 15) showed morphological differences in height, size of the leaf and other vegetative characters. The karyotypic study of these populations revealed differences of great significance i. e. one (Coll. no. 5) turned out to be a diploid taxon, while the other (Coll. no. 15) to be a tetraploid. The numbers reported here are 2n=36 and 72 in contrast to earlier reports of 2n=34 and 68 of Medvedeva (1936) and Skovsted (1935) respectively.

A diploid population (Coll. no. 5) analysed, had uniformly graded series of short to very short type of chromosomes. The somatic complement had 3 pairs with median, 12 pairs with sub-median and 3 pairs with sub-terminal centromeres. No chromosome with secondary constriction or satellite was observed (Figs. 5 and 5-A).

A tetraploid population (Coll. no. 15) analysed also showed uniformly graded chromosomes of the similar types. There are 14 pairs of chromosomes with median, 21 pairs with sub-median and only one pair with sub-terminal centromeres. Secondary constriction and satellite have not been observed on any pair.

Though two populations show gross similarity in chromosome types, the number of pairs with median, submedian and sub-terminal centromere differ markedly in the two.

Meiotic study showed the presence of 18 and 36 bivalents at diakinesis and metaphase I respectively (Figs. 9 and 10) contradicting the earlier report of n=17 by Skovsted (1935).

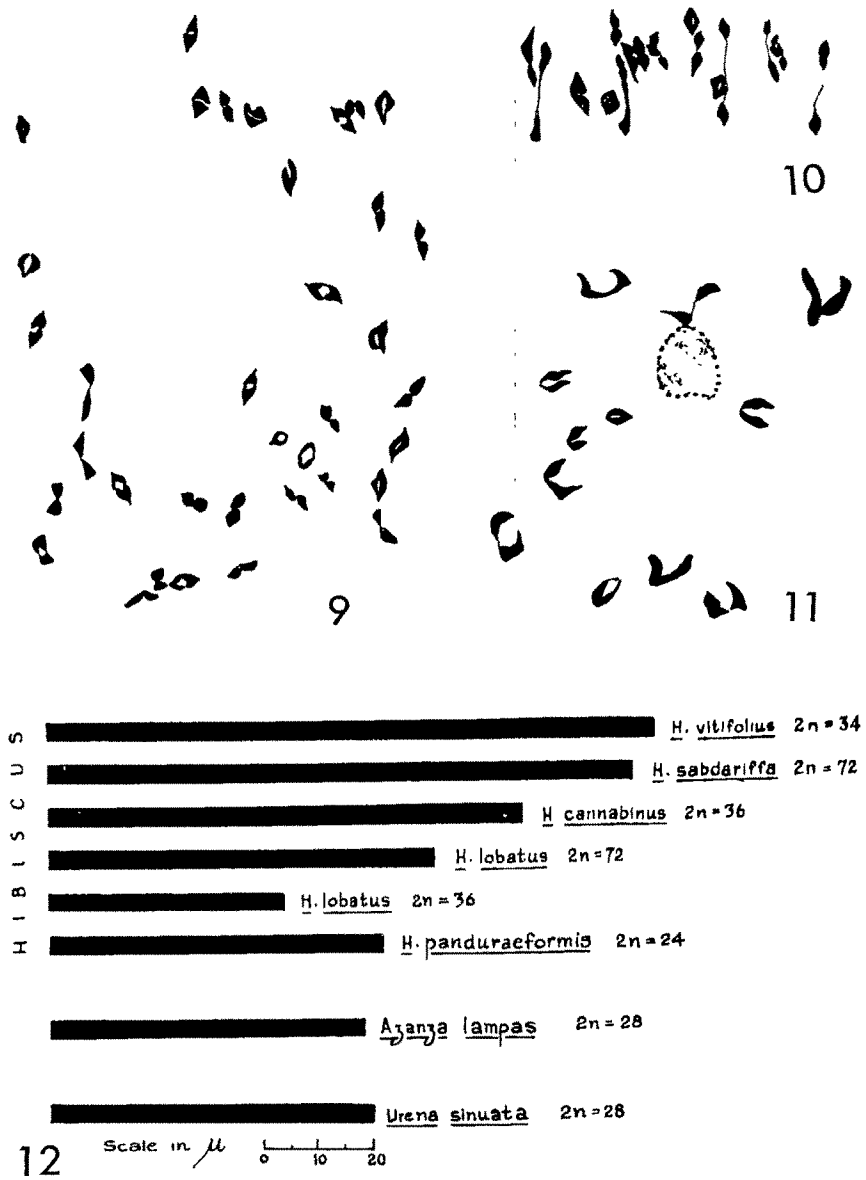
5) *H. panduraeformis*:

Absolute length —63.6 μ; Range —3.39 μ to 1.81 μ.
$2n=24=D_4+E_{16}+E_2'+H_2$

The earlier report 2n=24 (Skovsted 1941) is confirmed in the present study. No detailed account of the karyotype is available. The analysis reveals the presence of medium to short type of chromosomes, exhibiting symmetrical nature of the complement. But for two pairs of chromosomes, all the rest are having sub-median

centromeres. One pair of chromosome (E. type) has secondary constriction on the long arm.

No account of meiotic study could be traced from the available literature. The presence of 12 distinct bivalents is in full agreement with the somatic number reported



Figs. 9–8. 9, diakinesis of *H. lobatus* (n=36) 10, metaphase I, *H. lobatus* (n=18). 11, diakinesis, *H. panduraeformis* (2n=12). 12, histogram showing the total length of chromatin matter within the complement.

here (Fig. 11). One bivalent is found in the vicinity of nucleolus, supporting the role of chromosome with the secondary constriction, as nucleolar organiser.

Azanza

6) *Azanza lampas* (Cav.) Alef.:
=*H. lampas* Cav.
=*Thespesia lampas* Dalz. and Gibs.
=*Thespesia macrophylla* Blume:
Absolute length —59.4 μ; Range —3.06 μ; to 1.44 μ.
$2n=28=E_{14}+F_4+G_2+H_6+I_2$

No record of chromosome number or karyotypic study could be traced for the genus. The chromosome complement of the somatic tissue consists of 28 chromosomes distributed in 5 types. The complement is without any chromosome with satellite or secondary constriction (Figs. 7 and 7-A). 10 pairs with sub-median, 3 pairs with sub-terminal and 1 pair with median centromeres are observed.

Urena

7) *Urena sinuata* Linn.
Absolute length —61.7 μ; Range —2.97 μ to 1.61 μ.
$2n=28=E_{20}+E_2^{s'}+H_4+H_2^s$

2n=28 reported here confirms the earlier reports. The complement consists of uniformly graded series from medium to short type, having sub-median centromeres. The karyotype reveals the presence of one pair of chromosome with secondary constriction and another pair with satellites on their short arms. Marked differences are noticed in the karyotype analysed here and that of Hazra and Sharma (1971). Analysis of different populations within the geographical range of the species, is likely to reveal the presence of cytotypes. Occurrence of two darkly stained bodies within the complement confirms the existence of similar bodies in meiotic study, by Hazra and Sharma (1971). They probably represent the supernumerary or β-chromosomes.

Discussion

The family has received pronounced attention by many workers (Devie 1933, Skovsted 1935, 1941 Medvedeva 1936, Yongman 1931, Menzel and Wilson 1961, 1963, Roy and Sinha 1961, Sharma and Sharma 1962, Adhikary 1963, Hazra and Sharma 1971a, and 1971b, Kachecheba 1972, etc.). The data on the chromosome numbers for various genera is so much varied that no definite conclusion regarding the basic number for the family can be drawn. It is suggested by some earlier workers (Davie 1933, Hazra and Sharma 1971) that the number 7, is more prevalent and deep seated in majority of genera, should be regarded as the basic number for the family from which other numbers (7, 8, 9, 15, 17) have evolved.

Three genera viz. *Hibiscus*, *Azanza* and *Urena* considered in the present work show, in somatic complements a range of 2n numbers from 24, 28, 36 and 72 which are multiple of 6, 7 and 9. While, the number 34 is derived from either 7 or 9. Hazra and Sharma (1971) emphasized in their study of the genus *Thespesia*, that the

primitive basic number for the family could be 6. A similar situation is also encountered in the species *H. panduraeformis* (2n=24). This leads us to believe that 6 can be the primitive basic number for the family which is found in more than one genera. Many other numbers, reported for different species of the genus are derived from the latter. This suggests the existence of divergent lines of evolution among the genera and species of the family. Devie (1933) also subscribed to the polyphyletic view point and visualised the origin of different genera from the ancestral form (not mentioned) from the basic number 7 and not 6 as suggested here.

The gross similarity of the karyotypes studied, ascertains the inclusion of these three genera in the natural assemblage of the family.

Of the five species of *Hibiscus* analysed, *H. panduraeformis* seems to be more primitive, for it has 2n=24 chromosomes in the complement, containing long chromosomes with sub-median or median centromeres, only one pair of long chromosome (E.-type) has secondary constriction on long arm. Though, gross similarity in karyotypes is observed in the two species viz. *H. vitifolius* and *H. cannabinus* they differ from each other in chromosome number, pairs having secondary constriction or satellite and the total chromatin length. These serve as markers for specific distinction of the two. *H. vitifolius* having 2n=34 is more advanced than *H. cannabinus* (2n=36), for the former has more pairs with secondary constrictions. In contrast to the species considered above *H. lobatus* and *H. sabdariffa* show the presence of karyotypes having medium to very short types of chromosomes. The size difference in both is of graded nature, and both show higher degree of ploidy levels. The presence of 2 pairs having tandom satellites in *H. sabdariffa* and its absence in *H. lobatus* shows that the former is slightly more advanced than the latter.

2n=28 is observed in the species *Azanza lampas* and *Urena sinuata*. In spite of gross similarities, the differences between the two are well marked. *Azanza lampas*, which is studied here for the first time, reveals the primitive nature of karyotype consisting of majority of chromosomes with median centromeres and without any satellite or secondary constrictions. This supports the view of morphologists to maintain a separate status of this species (i. e. in genus *Azanza*) and be considered intermediate between *Thespesia* and *Hibiscus* (Rakshit and Kundu 1970). *Urena sinuata* is comparatively more advanced in having 1 pair with satellite and 1 pair with secondary constriction. Its advance nature is further evidenced by the presence of 2 supernumerary chromosomes in the somatic complement.

Detailed analysis of the karyotypes show distinctive differences in minute details, suggesting thereby that the structural alternations, have played a greater role in speciation. It can be concluded though guardedly, that ploidy (euploidy and aneuploidy) accompanied by structural changes in different combinations may be responsible for the course of evolution within the family.

Summary

Three genera viz. *Hibiscus, Azanza* and *Urena* are considered for the present investigation.

Of the five species of *Hibiscus* analysed *H. panduraeformis* seems to be most

primitive while, *H. vitifolius* and *H. cannabinus* are slightly more advanced. *H. lobatus* and *H. sabdariffa* have medium to very short types of chromosomes and show higher ploidy levels, and thus be considered more advanced than others.

The problematic status of the genus *Azanza* is reviewed in the light of our findings, which ascertains the maintainance of distinct generic status and be placed inbetween *Thespesia* and *Hibiscus*.

The more evolved nature of *Urena sinuata* is ascertained by the presence of 2 super-numerary, secondarily constricted and satellited chromosomes in the complement. Existence of cytotypes within the circumscription of species is suspected.

Regarding the basic number for the family, it is concluded that 6 can be the basic number, found in more than one genera; and evolution must have taken place along divergent lines among the genera and species. The course of evolution within the family might be through ploidy accompanied by structural changes in different combinations.

Acknowledgement

Thanks are due to Prof. C. H. Pathak, Head, Department of Botany and Dr. S. D. Sabnis, Reader in Botany, M. S. University of Baroda for constant encouragement during the course of the work; to Dr. (Mrs.) A. Sharma, Reader in Botany, Calcutta University, Calcutta and Professor M. S. Chennaveeraiah, Head, Department of Botany, Karnatak University, Dharwar for their help and suggestions in the preparation of the manuscript.

Literature cited

Adhikary, A. K. 1963. Cytotaxonomical studies in some species of *Sida* (Malvaceae). Trans. Bose. Res. Inst. **26**: 59–83.

Chennaveeraiah, M. S. and Subbarao, G. 1965. Chromosome number and karyomorphology in *Hibiscus cannabinus* and *H. sabdariffa*. Jour. Karnatak Univ., Sci. **9**: 78–87.

Darlington, C. D. and Wylie, A. P. 1955. Chromosome Atlas of Flowering Plants. (II Ed.). Allen. and Unwin Ltd. London.

Devis, J. H. 1933. Cytological studies in the Malvaceae and certain related families. J. Genet **28**: 33–67.

Hazra, R. and Sharma, A. 1971a. Chromosome studies in different species and varieties of *Sida* with special reference to accessory chromosomes. Cytologia **36**: 285–297.

— and — 1971b. Further studies on cytotaxonomy of *Malvaceae*. Genet. Iber. **23**: 145–166.

Kachecheba, J. L. 1972. The cytotaxonomy of some species of *Hibiscus*. Kew Bulletin **27**: 425–433.

*Medvedeva, G. B. 1936. Karyological review of 15 species of the genus *Hibiscus*. Jour. Bot. de. L'. URSS. **21**: 533–550.

Menzel, M. Y. and Wilson, F. D. 1961: Cytotaxonomic relationships in *Hibiscus*. Sect. Furcaria (Abstr.) Amer. Jour. Bot. **48**: 535.

— and — 1963. Cytotaxonomy of twelve species of *Hibiscus* Sect. Furcaria. Amer. Jour. Bot. **50**: 262–271.

Raizada, M. B. 1966. Nomenclatural change, in Indian plants. Indian Forester. **92**: 229–339.

Rakshit, S. C. and Kundu, B. C. 1970: Revision of the Indian species of *Hibiscus*. Bull. Bot. Surv. India. **12**: 151–175.

Rao, M. B., V. N. 1935. Chromosome numbers in two species of *Hibiscus* (*H. sabdariffa* and *H.*

cannabinus). Curr. Sci. **4**: 162 and 175.
Roy, R. P. and Sinha, R. P. 1961. Meiotic studies in some malvacious species. Curr. Sci. **30**: 26–27.
Sharma, A. K. and Sharma, A. 1962. Polyploidy and chromosome evolution in *Hibiscus*. La Cellule **62**: 182–300.
— and — 1972. Chromosome Techniques. Theory and Practice (II Edition) Butter Worth and Co., London and University Park Press Baltimore.
Skovsted, A. 1935. Chromosome number in the Malvaceae I. J. Genet. **31**: 263–296.
— 1941. Chromosome numbers of the Malvaceae II. Compt. Rend. Labor. Carlsberg **23**: 195–242.
Stebbins, G. L. Jr. 1964. Variation and Evolution in Plants. Reprinted. (Indian Edition) Oxford Book, Company, Calcutta.
Tjio, J. H. 1948. The somatic chromosomes of some tropical plants. Hereditas **34**: 135–146.
Tjio, H. A. and Levan, A. 1950. The use of oxiquinoline in chromosome analysis. Ann. Estae. Exp. Aula Dei. **2**: 21–64.
Yongman, W. 1927. Studies in the cytology of Hibisceae. Ann. Bot. **41**: 755–778.

---

* Not consulted in original.