MATERIALS AND METHODS

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Taxonomy :

Different populations of the plant species selected for the present work were collected from different localities (Table 1) and habitats. Along with the collection of plant specimens and mature seeds, observations regarding their habit, average size, state in which observed, colour and/or smell of the flower and other striking morphological peculiarities were recorded. A separate voucher number was given for each population. Tentative identifications were confirmed after dissection of various parts and by referring the standard floras available. Herbarium sheets of these were also prepared following Lawrence (1951), and they are deposited as voucher specimen in the departmental herbarium. Mature seeds of all these populations were grown in the Botanical garden under identical conditions, for checking the variations within the populations, to detect the presence of ecological races and for selecting suitable buds for meiotic studies.

The morphological data concerning variation in

different populations of a species were used for the preparation of polygraphs following Hutchinson's technique (1936) modified by Löve and Nadeau (1961). For this, important morphological characters were selected and three index values were assigned to the morphological variations observed. 'Polygraph key' was prepared in the shape of a wheel with radiating spokes, each spoke representing one morphological character. The distance along the spoke represents the variations of that particular character as described in the characters of each taxon. In plotting the polygraph, marks on each spoke on a particular position (or variation of a character) was made. Then these points were joined to form a polygonal graph or polygraph. Polygraphs of different populations of the same taxon or related taxa were superimposed to construct a polygram revealing the variability or consistency of characters within the taxa under consideration. Number in the parenthesis represents the collection number of a particular population.

Cytology :

Mature seeds for the cytological investigations were collected or obtained from different localities in

Gujarat, Madhya Pradesh, Uttar Pradesh and Karnataka (Table I).

Table I.

Tribe : Hibisceae

Table showing taxa, collection number, source and/or locality.

Sr. No.	Taxa	Collection number	Source or locality
1.	Hibiscus sabdariffa	07	Baroda
2.	" " var. 1-4 red	60	Dharwar
3.	" " var. 1-9 red	61	"
4.	" " var. 9-green	62	"
5.	" " var. 1-1-green	63	"
6.	Hibiscus cannabinus	02	Rajpipla
7.	" "	14	Baroda
8.	" "	22	Pavagadh
9.	" "	24	Dilavari forest
10.	Hibiscus vitifolius	04	Rajpipla
11.	" "	21	Pavagadh
12.	" "	33	Dangs forest

Contd.....

Table I Contd.

Sr. No.	Taxa	Collection number	Source or locality
13.	Hibiscus mutabilis	44	Dehra Dun
14.	Hibiscus trionum	47	Kolar
15.	Hibiscus lobatus	05	Rajpipla
16.	" "	15	Khedbrahma
17.	" "	17	Pavagadh
18.	" "	25	Dilavari forest
19.	" "	46	Dangs forest
20.	Hibiscus hirtus	54	Baroda
21.	Hibiscus ovalifolius	28	Harani
22.	" "	56	Vasad
23.	Hibiscus panduraeformis	03	Rajpipla
24.	" "	09	Dabhoi
25.	" "	26	Samardha forest
26.	" "	37	Baroda
27.	Hibiscus caesius	43	Chhota-udepur
28.	" "	55	Vasad
29.	Abelmoschus manihot	08	Rajpipla

Contd.....

Table I Contd.

Sr. No.	Taxa	Collection number	Source or locality
30.	Abelmoschus angulosus	52	Dehra Dun
31.	Azanza lampas	10	Damaras
32.	" "	12	Chhotaudepur
33.	" "	31	Rajpipla
34.	" "	32	Khedbrahma
35.	" "	51	Dehra Dun
36.	Thespesia populnea	49	Baroda
37.	" "	50	Pavagadh
		Tribe : Ureneae	
38.	Malchra capitata	36	Khedbrahma
39.	" "	38	Baroda
40.	Urena lobata	27	Dilawari forest
41.	" "	29	Harni
42.	" "	41	Baroda
43.	" "	48	Dehra Dun
44.	Pavonia zeylanica	40	Baroda
45.	" "	57	Vasad
46.	Pavonia patens	16	Kutch

For mitotic preparation, seeds were germinated in petri dishes on moist filter paper at room temperature. Where germination was difficult due to hard seed coat, seeds were treated with conc. H_2SO_4 for 1 to 2 minutes and thoroughly washed in running tap water to enhance seed germination (Rao, 1947). Different pre-treatment chemicals such as 8-hydroxyquinolin (Tjio and Levan, 1950), Paradichlorobenzene (Meyer, 1945) and aesculin (Sharma and Sarkar, 1955) were tried on excised root-tips to get satisfactory preparations of somatic chromosomes. Out of these, saturated solution of paradichlorobenzene for 1½ hour in cold temperature (15° to 20°C) between 9-25 to 9-35 a.m. gave the best results for most of the taxa. Root tips of Abelmoschus manihot gave good results in saturated solution of aesculin for 10 minutes in cold (15° to 20°C) at 9-25 a.m. Pretreated root-tips were washed and fixed in acetic - alcohol mixture (1 : 3) for one hour. Then they were transferred to 2% aceto-orcein and N HCl mixture (9 : 1) and heated on a flame for few seconds until the vapours appeared and kept in that acid-dye mixture for 15 minutes for cooling. Finally the tips were squashed

in 1% aceto-orcein solution, applying uniform pressure over the cover glass. Selected slides were sealed with wax. This method provided satisfactory results, since constrictions (primary and secondary) were exaggerated and chromosome arms were shortened, thus facilitating the counting of chromosome number and to study the morphology of chromosomes. Overlapping was also avoided to a large extent. Preparations were made from different collections, but only the best plates were selected for drawings and photographs.

Difficulty was encountered in studying meiotic divisions which were obtained from both fresh and fixed flower buds. The fixed materials showed the best results in most of the cases. The flower buds were fixed between 1-30 to 2-30 p. m. in modified Carnoy's fluid (6 : 3 : 1) for at least 24 hours to clear the cytoplasm. In Thespesia populnea and Azanza lampas only the anther mass was fixed in the fixative after several washes in the same to get good preparations. Before smearing in 2% aceto-carmine, the anthers were transferred in 45% acetic acid for at least 1 hour.

The slides were made permanent following Celariar's

(1956) butyl alcohol schedule and mounted in euparal.

Drawings were made at table-level on Steindorff microscope using E. Leitz-Wetzlar camera lucida apparatus with removable filters, using x 15 or x 30 eye pieces and x 100 oil immersion or x 120 apochromatic objectives. Photographs were taken by Exakta (Varex) camera using the ORWO-35 mm black and white film.

The apparent pollen fertility was estimated on the basis of stainability of pollen in equal proportion of acetocarmine and glycerine mixture (Muntzing's solution, see Sharma and Sharma, 1972).

In order to study the karyotypes several slides were made for each taxon, from which two or three good metaphase plates were selected for drawings. After drawing, chromosomes were numbered,and measured by thread in millimeters. The chromosomes were matched into pairs and mean values of the long and the short arms of each pair were calculated. Then length of the chromosome arms were converted from millimeters to microns by multiplying the value got by calibration of the

microscope in different magnifications. The satellites and β-chromosomes were not measured. To decide the centromeric position, arm ratios $(R_1) = \frac{\text{short arm}}{\text{long arm}}$, $(R2) = \frac{\text{long arm}}{\text{short arm}}$; (Adhikary, 1974) and centromeric index $(F\%) = \frac{\text{length of short arm}}{\text{Total length}} \times 100$ were calculated. For comparing chromosome size and to decide the (graded) nature of the karyotype, relative length $\left(\frac{\text{Length of the individual chromosome}}{\text{Length of the longest chromosome}} \times 100\right)$ was used. For deciding the symmetrical or asymmetrical nature of karyotype, total form percentage (TF%) = $\frac{\text{Total sum of the short arm}}{\text{Total sum of chromosome length}} \times 100$ and ratio of longest to the shortest pair (L/S) were calculated. F%, relative length and TF% were calculated following Huziwara (1958). A TF% of 50 indicates that all the chromosomes have median centromeres and therefore, absolute symmetry of the karyotype, whereas a TF% of zero indicates that all the chromosomes have terminal centromeres so the karyotype becomes completely asymmetrical. High value of the ratio of longest to the shortest pair indicates asymmetry of the karyotype.

Most of the calculations were first computerised and later the values were varified. The calculations were

programmed in FORTRAN-IV language and run on ICL-1901 computer. FORTRAN-IV (Formula translation) is the machine language which the computer understands(Refer Vol. II).

The idiograms were first drawn on the graph paper in decreasing order of length from left to right. The ends of long arms were directed downwards lying on the same abscissa. Then the drawings were transferred to the drawing paper. The width of the chromosome and the gap of the primary and secondary constrictions were maintained uniformly throughout. The scale is represented in microns by the side of the idiogram. The type of each chromosome is mentioned below. In camera lucida drawings and in idiograms, satellited or secondarily constricted chromosomes are drawn by outlines.

Total chromatin length of a diploid set of chromosomes is represented by histograms. The scale is given by the side of histogram.