PART I

MATERIALS AND METHODS

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Materials for the present investigation were collected and obtained from different localities in India and abroad (Table 1). In the field, 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. For each collection, voucher number was given. Tentative identifications made, were later confirmed after dissecting various parts and by referring to the standard floras available. Herbaria were prepared following Lawrence (1951). Vouchers are deposited in the departmental herbarium.

Table 1. Taxa investigated.

Taxa	Collection Number	Locality or Source
INDIGOFERA Linn.		
Indigofera tinctoria	1	Dangs forest
" "	2	Harani
" "	3	Aurangabad
" "	4	Ambaji

Taxa	Collection Number	Locality or Source
Indigofera tinctoria	5	Sabarkantha
Indigofera astragalina	6	Dangs forest
" "	7	Pavagadh
" "	8	Coimbatore
Indigofera oblongifolia	9	Sabarkantha
" "	10	Gir forest
" "	11	Baroda
Indigofera trita	12	Gir forest
" "	13	Dehradun
" "	14	Pavagadh
" "	97	Kolar
Indigofera glandulosa	15	Gir forest
" "	16	Pavagadh
" "	17	Aurangabad
" "	85	Khedbrahma
Indigofera linifolia	19	Dangs forest
" "	20	Sabarkantha
" "	21	Pavagadh
Indigofera linifolia var. campbelli	18	Gir forest
" " "	62	Aurangabad
Indigofera linnaei	22	Bangalore

	Taxa	Collection Number	Locality or Source
	Indigofera linnaei	23	Pavagadh
	" "	24	Dangs forest
	Indigofera cordifolia	25	Baroda
	" "	60	Sabarkantha
	" "	26	Dangs forest
	Indigofera angulosa	27	Khedbrahma
	" "	63	Gir forest
	Indigofera hochstetteri	28	Khedbrahma
	Indigofera trifoliata	29	Savli
	" "	30	Baroda
	Indigofera duthei	59	Aurangabad
*	Indigofera hirsuta	102	Kenya
	" "	57	I.A.R.I., New Delhi.
*	Indigofera colutea	58	Coimbatore
*	Indigofera spicata	96	Zambia
	Indigofera subulata	105	Kenya
*	Indigofera arrecta	104	Kenya
*	Indigofera amblyantha	111	Kew Gardens
*	Indigofera heterantha	112	Kew Gardens
*	Indigofera vicioides	103	Kenya

	Taxa	Collection Number	Locality or Source
	DESMODIUM Desv.		
	Desmodium gangeticum	48	Dangs forest
	" "	49	Khedbrahma
	" "	56	Gir forest
	" "	73	Baroda
	" "	74	Dehradun
	Desmodium dichotomum	50	Khedbrahma
	" "	51	Gir forest
	" "	87	Dangs forest
	Desmodium triflorum	55	Baroda
	" "	53	Ambaji
	Desmodium rotundifolium	52	Dangs forest
	Desmodium laxiflorum	54	Pavagadh
	Desmodium velutinum	75	Chhotaudepur
	" "	77	Dehradun
*	Desmodium salicifolium	95	Zambia
	Desmodium heterocarpon var. strigosum	98	Ratanmahal
*	Desmodium intortum	106	Argentina
	Desmodium distortum	109	Argentina
*	Desmodium uncinatum	108	Brazil
*	Desmodium sandwicense	107	Hawaii
*	Desmodium elegans	114	Kew Gardens

	Taxa	Collection Number	Locality or Source
	DENDROLOBIUM (Wt. & Arn.) Benth.		
*	Dendrolobium triangulare	78	Coimbatore
	ALYSICARPUS Desv.		
	Alysicarpus procumbens	31	Dangs forest
	" "	32	Devgadh Baria
	" "	83	Baroda
	Alysicarpus monilifer	37	Devgadh Baria
	" "	38	Baroda
	" "	39	Pavagadh
	" "	67	Aurangabad
	Alysicarpus vaginalis	35	Baroda
	" "	36	Pavagadh
	" "	64	Vasad
	" "	65	Dehradun
	" "	66	Dangs forest
	Alysicarpus ovalifolius	93	Zambia
	" "	116	Baroda
	Alysicarpus glumaceus	91	Zambia
	" "	110	Kenya
	Alysicarpus heyneanus	33	Vasad
	" "	34	Dangs forest

Taxa	Collection Number	Locality or Source
Alysicarpus heyneanus	68	Chhotaudepur
" "	45	Harani
Alysicarpus styracifolius	40	Dangs forest
" "	41	Timbi
" "	42	Khedbrahma
* Alysicarpus rugosus	92	Zambia
Alysicarpus longifolius	43	Timbi
" "	44	Dangs forest
" "	82	Baroda
" "	86	Sabarkantha
" "	84	Rajpipla
Alysicarpus tetragonolobus	46	Pavagadh
" "	47	Dangs forest
" "	88	Gir forest
" "	72	Aurangabad
Alysicarpus wallichii	69	Dehradun
" "	70	Dangs forest
" "	71	Gir forest
" "	89	Chhotaudepur
" "	90	Rajpipla
Alysicarpus bupleurifolius	101	Timbi

* The species marked with asterisks fail to germinate or flower in Baroda conditions. Hence, meiosis of these species could not be carried out.

A short morphological description based on actual observations is given for each species. Hutchinson's technique (1936) modified by Love and Nadeau (1961) was adopted to draw polygram based on important morphological variations observed in different populations of a species or allied taxa. Three index values were assigned to morphological variations observed in each character. To construct a polygraph, a key is prepared in the shape of a wheel with radiating spokes. The centre of the wheel is represented by a point. Each spoke denotes a morphological character and the distance along the spoke represents the variation of that particular character. In plotting the polygraph, a mark on each spoke at a particular point was made. These points were later joined to form a polygonal graph or polygraph. Polygraphs of different populations of the same taxon or allied taxa were interpolated to construct a polygram, revealing the variability or consistency of characters within taxa under consideration. In the polygram, the polygraphs of different populations are drawn in different colours. In the present study, only polygraphic key and polygrams are represented.

Due to the presence of hard seedcoat, the seeds were treated with conc. H_2SO_4 for 2 - 3 minutes, and then thoroughly washed in running tap water, to enhance germination (Rao, 1947).

Treated seeds were placed in a petridish on a moist filter paper at room temperature. The young seedlings were then transferred to the pots in the Botanical garden, where they were grown in identical conditions, to detect the presence of ecological races, if any.

The mitotic study was made following Tjio and Levan's (1950) oxyquinoline aceto-orcein squash method. This method proved to be most satisfactory since the constrictions were exaggerated and chromosome arms were suitably shortened. The excised root tips from the seedlings were treated with 0.002 M 8-hydroxy-quinoline for 1 - 2 hours at 12-15°C. Pretreated root tips after washing were fixed in acetic - alcohol mixture (1 : 3) for one hour. These root tips after hydrolysis in a mixture of 2% aceto-orcein and 1N HCl (9 : 1) were squashed in 1% aceto-orcein. The slides were sealed with wax and they remained good for a week. For karyotypic study of different collections of a species, a number of preparations were made from root tips. The divisions in the root tips were found to be maximum between 9.45 A. M. and 10.30 A. M. and it mostly depends on the diurnal changes. A comparative karyotypic study was carried out to ascertain particularly the chromosome number and morphological details. Only the best

plates among the freshly prepared slides, were selected for drawing and photography.

All drawings were made at table level on Steindroff microscope using E. Leitz-Wetzler camera lucida apparatus with removable filters, using 15 X or 30 X eye piece and 120 apochromat objective. Photomicrographs were taken with 35 mm Exacta (Varex) camera using 10 X eye piece and 120 apochromat objective and later enlarged to a suitable size.

The chromosomes in the camera lucida drawings were numbered and then measured by thread in milimeters. The measurements in milimeters were converted into microns by comparing them with the drawn scale of stage micrometer under the same magnification. Long arm, short arm and secondarily constricted portions were measured separately. The gap caused by primary or secondary constriction was not taken into consideration because they were affected by pretreatment. All the measurements of chromosomes in a complement were tabulated, then the homologous chromosomes were paired. The mean values for long arm, short arm and secondarily constricted portions for the pair were calculated. The mean length of the chromosomes in a complement is also calculated.

To decide the centromeric position, arm ratios were calculated (Table 2) following Adhikary (1974).

Table 2. Arm ratios and position of centromere

$\frac{\text{Short arm}}{\text{Long arm}}$ (R_1)	$\frac{\text{Long arm}}{\text{Short arm}}$ (R_2)	Position of the centromere	Notation
1.00	1.00	Median	M
0.33	3.00	Submedian	SM
0.99 - 0.61	1.01 - 1.63	Nearly median	nm
0.60 - 0.34 and 0.32 - 0.23	1.64 - 2.99 and 3.01 - 4.26	Nearly submedian	nsm

Chromosome measurements due to pretreatment and various other factors, might vary in different populations. This was minimized by calculating the relative length, expressed as percentage length of chromosomes to the longest chromosome pair (Huziwara, 1958; Kapoor and Löve, 1970).

For deciding the symmetry of a karyotype "Total Form percentage" (TF%) i.e. total sum of short arms X 100/total sum of all the chromosomes (Huziwara, 1958) and ratio of longest to the shortest pair (L/S) were calculated. A TF% of 50 indicates that all chromosomes have median centromeres and therefore, absolute symmetry of the karyotype, whereas a TF% of zero indicates that all chromosomes have terminal centromeres, so the karyotype becomes completely asymmetrical

(Kapoor and Löve, 1970). The high L/S ratio is also indicative of apparent asymmetrical nature of the karyotype (Kapoor and Löve, 1970).

Idiograms were first drawn on the graph paper in decreasing order from left to right, the ends of long arms directed downwards lying on the same abscissa. Then the drawings were transferred to the drawing paper. The width of the primary and secondary constrictions, were maintained uniformly throughout the work. The uniform gap maintained for constrictions does not add to the length of the chromosomes in the idiograms. The scale is represented by the side of the idiograms. The type is mentioned below each chromosome. In camera lucida drawings and idiograms, the chromosomes with satellites or secondary constrictions are drawn in outlines. The chromatin length of a diploid set of chromosomes is represented by histograms. The scale is given below the histogram.

For meiotic study flower buds of suitable size were fixed in Carnoy's fixative (6 parts of alcohol : 3 parts of chloroform : 1 part of acetic acid) between 9 A. M. and 11 A. M. These were later transferred to fresh fixative, which remained good for a month.

In majority of the cases flower buds were fixed from the experimental garden. After 24 hours, the buds were used for smear preparation in 2% aceto-carmine. The excess stain was removed by placing the slide between folds of the blotting paper. A little pressure was applied on the cover glass to get uniform spreading of pollen mother cells. Then the slides were sealed with wax and kept overnight for the stain to intensify.

The mitotic and meiotic preparations were made permanent following Celariar's (1956) Butyl alcohol schedule and mounted in euparol.

The pollen fertility was estimated on the basis of stainability of pollen in Glycerine and aceto-carmine (1 : 1) mixture. The estimate is based on examination of number of pollen grains in several microscopic fields. Only well-inflatened, uniformly stained grains were scored as fertile ones.

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