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*MATERIALS AND  
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## **MATERIALS AND METHODS**

### **Floristics:**

The populations of different taxa mentioned in the present work were collected from different localities of this semi-arid region. Number of field excursions, each of 1 week's duration were organised. Localities like Khavda in north zone, Anjar and Gandhidham in east zone, Mandvi and Mundra in south zone, Nakhatrana, Nalia and Narayan Sarovar in west zone and Bhuj in central zone were selected for base camps.

More frequent visits were made to above localities and the plant species were collected at various stages i.e., vegetative and reproductive. Field observations on habitat, rock & soil types and water resources in the zone were

recorded. Moreover, notes on associates, habit, state in which observed, colour and/or smell of flowers and other striking morphological features were noted. Photographs depicting different vegetational aspects at different localities were also taken. Plant taxa collected from different localities were given separate collection numbers.

The tentative field identifications were later on confirmed, in the laboratory by dissecting under stereo-microscope and by reference to various regional floras like Flora of British India (Hooker, 1879), The flora of Bombay Presidency (Cooke, 1905), Flora of Gujarat State (Shah, 1978), and Flora of the Indian Desert (Bhandari, 1990) and other available literature. Herbarium sheets representing different populations were prepared following Lowrence (1951), Santapau (1954) and Radford et al. (1974) and deposited in departmental herbarium.

Attempts have been made to adopt the most recent nomenclature. All the new names have been given on the authority of the latest floras. Only recently accepted names, basionyms and synonyms have been given in the text.

For, rare/restricted or otherwise interesting plants, morphological description based on the study of different populations is given. This is done with a view to give detailed account of variations existing in them. At the same

time endeavour has been to describe each plant species in depth so as to give exhaustive and complete morphological description.

### Cytology:

The mature seeds and suitable sized flower buds were collected for cytological work. Seeds representing different populations were germinated in petridishes on moist filter papers at room temperature. On certain occasions seeds were subjected to various treatments to enhance the seed germination.

Fresh and healthy roots, subjected to various pre-treatment; 8-Hydroxyquinoline, p-Dichlorobenzene, Colchicine and Esculine were tried for selecting the suitable pre-treatment chemical, which gave satisfactory results. Root-tips were collected between 9.15 and 10.15 a.m. Selected root-tips were treated with 0.002 M 8-Hydroxyquinoline or saturated solution of p-Dichlorobenzene for 2½ to 3 hrs at 10-12°C; while, chilled 0.2% Colchicine for 2-4 hrs at room temperature (Sharma and Sharma, 1972). Senra incana, Abutilon pannosum, Pavonia zeylanica, Fagonia indica, and Zygophyllum simplex gave very good results when treated with p-Dichlorobenzene. Colchicine was found to be suitable for Launaea resedifolia and Chascanum marrubifolium. Indigofera argentea, Schweinfurthia pterosperma and Capparis

cartilaginea gave satisfactory results when treated with 8-Hydroxyquinoline.

The mitotic study was made following Tjio and Levan's (1950) Oxyquinoline aceto-orcein schedule. Slides showing good metaphase plates were temporarily sealed with paraffin wax. For ascertaining the chromosome number and to study the chromosome morphology, a number of preparations were made.

Selected mitotic preparations showing good stages were used for Camera Lucida drawings and microphotographs. All drawings were made at table level on Steindorf's Research Microscope and Carl-Zeiss 'Amplival' Research microscope using E. Leitz-Wetzlar Camera Lucida apparatus at x1500, and x2160 magnifications. Microphotographs were taken on Zeiss-Research photomicroscope by using x1000 magnification and ORWO NP-55 black and white film. Both in Camera lucida and Idiogram drawings, satellited and secondary constricted chromosomes have been drawn in outline only.

Temporary slides were later on made permanent following Celariar's (1956) butyl alcohol-acetic acid schedule, and mounted in euparal.

For Karyotype analysis following values such as Relative length, TF %, TCL %, L/S and S% were calculated following Huziwara (1958), Kapoor and Love (1970) and Adhikary (1974) respectively.

$$\begin{aligned} \text{Relative length \%} &= \frac{\text{Total length of chromosome}}{\text{Total length of largest chromosome}} \times 100 \\ \text{TF \%} &= \frac{\text{Total length of short arm}}{\text{Total length of chromosome complement}} \times 100 \\ \text{TCL \%} &= \frac{\text{Total length of a chromosome}}{\text{Total length of a chromosome complement}} \times 100 \\ \text{L/S} &= \frac{\text{Length of longest pair}}{\text{Length of shortest pair}} \times 100 \\ \text{S \%} &= \frac{\text{Relative length of smallest chromosome}}{\text{length of longest chromosome of a complement.}} \end{aligned}$$

Fixed flower buds were used for meiotic smear preparations. Flower buds were fixed in Carnoy's fixative (6 parts Ab. Alcohol: 3 parts Chloroform : 1 part Acetic Acid) in between 9.30 to 11.30 a.m. for 24 hours and for longer duration preservations, buds were transferred into fresh Carnoy's fixative. However, before smearing, fixed material were transferred into 45% acetic acid for 30 minutes. The smear preparations were made in 1% aceto-carmin. In selected stages of meiotic preparations, observed abnormalities, were drawn at x1500 magnification using camera lucida apparatus. Microphotographs were also taken to illustrate the same. Meiotic stages and abnormalities were drawn by applying above mentioned procedure.

The apparent pollen fertility was estimated on the stainability of pollen in Muntzing Solution (1:1 proportion at glycerol and 1% aceto-carmin).

**Micromorphology:**

Epidermal peels were taken from fixed leaves, leaflets or herbarium specimens. Leaves were boiled in distilled water in a hot bath for 3-4 min. In some cases, where peeling was difficult maceration was done by boiling in Jeffrey's fluid (1:1 proportion of 10% Nitric acid and Chromic acid). The epidermal peels were cleaned with soft brush. In order to remove trichomes, occasionally leaves were scrubbed on musline cloth or zero number sandpaper. The cleared peels were stained in Delafield's haematoxyline (1%) and thoroughly washed in water and mounted on slides in glycerine jelly.

For each species, observations regarding cell shape, frequency, stomatal types, stomatal frequency etc., were determined on the basis of the study of 10 fields under microscope. Stomatal index value was calculated by following Salisbury (1927, 1932); while, absolute veinislets number and palisade ratio values were calculated following Gupta (1961).

Drawings, representing epidermal features were drawn at table level on Steindorff's Research Microscope using Camera lucida apparatus at x 60 and x 30 magnifications. Photomicrographs were also taken directly from the slides, using incandescent light, yellow filter and ORWO-NP-55 black

and white film.

For description, terminologies of Metcalf and Chalk (1950), Uphof and Hummel (1962), Pyne (1979) for trichomes and Metcalf and Chalk (1950), Metcalf (1961) Stace (1965) and Inamdar et al (1986) for stomata have been adopted.

### **Seed Germination**

Seed populations which posed germination problems were subjected to physical as well as chemical treatments, in order to promote germination.

Mature seeds were surface sterilised with 1% mercuric chloride for 2 min. and washed thoroughly with distilled water and blotted. Each petriplate contained 20 seeds on moistened filter paper and each treatment was replicated 3 times. The rate of emergence was noted at 24 hrs interval for a period of 120 hrs.

### **Seed treatments**

**A) Soaking :** Seeds were exposed for ( $70^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) water 10 min. and were then soaked in distilled water for 24 hrs. These seeds were then placed in petriplates containing moist filter paper.



- B) **Acid scarification :** Seeds were scarified with conc.  $H_2SO_4$  for 30 sec. to 120 sec. and washed thoroughly with water, before placing in petriplates.
- C) **Treatment with germination stimulants:** A number of chemicals such as thiourea (0.25%, 0.5%, 1%) and Gibberellic acid, Indole-acetic-acid, were tried individually and in combination (5, 10, 20, 30 ppm) to enhance germination of acid scarified seeds.