CHAPTER 2

MATERIALS AND METHODS

2.1. Climatic Data

Climatic data for Baroda were obtained from the Meteorological Observatory of the M. S. University of Baroda.

2.2. Microclimatic Data

Microclimatic data for L. V. Palace compound were recorded for the years 1978 and 1979. The same for the University Campus were obtained from the observatory.

2.3. Soil Analysis

Soil samples were collected from the top 20 cm layer from different study sites, labelled properly, and brought to the laboratory in polythene bags. They were then air-dried, ground in a porcelain mortar, passed through a 1 mm sieve, and stored in stoppered glass bottles.

Standard methods as given by Piper (1944), Jackson (1962), Misra (1968) and Pandeya et al. (1968) were employed for the analysis.

The analysis of the samples was carried out in the laboratory of the soil Science Department, Agriculture College, Navsari.

Soil pH - was determined by electrometric method using pH meter.

Organic matter - was roughly estimated by loss on ignition method.

Organic carbon - was calculated by coloriemetric method.

Total Nitrogen - was determined by Kjeldahl's method.

Carbonate, Bicarbonate and Chloride - were determined by titration method.

Exchangeable Calcium and Magnesium - were determined by titration method using Ammonium acetate extract.

Exchangeable Sodium and Potassium - were determined by Flame Photometry using Ammonium acetate extract.

Electrical Conductivity (EC) and Total Soluble salts - EC was determined with the aid of a conductivity meter and Total Soluble Salts were estimated from the value of EC.

2.4. Anatomical Studies

Material for anatomical studies was collected from the study sites and was fixed in FAA (Formalin-Acetic-Alcohol). Microtome sections 20-25 u thick, of the stem, root, petiole and leaf were cut and stained with safranin and fast green. Wherever convenient, hand-sections were also taken and stained with safranin and fast green.

2.5. Stomatal Index

Fresh leaves after being detached from the plants were

brought to the laboratory with their basal part dipped in water. The epidermis was then peeled off, fixed in absolute alcohol, and mounted in a drop of 10% glycerine.

The peeling off of the epidermis was found difficult especially in leaves of Abutilon ramosum and Rivinia humilis. In such cases a thin film of 'Quick fix' adhesive was uniformly applied on the surface of the leaf.This was then allowed to dry up, after which the film of 'Quick fix' carrying the impression of the leaf surface was stripped off, mounted dry on a slide and covered under a coverslip.

The total numbers of stomata and epidermal cells, occurring per unit area were determined, and then the stomatal index was calculated by using the formula :

$$I = \frac{S}{S + E} \times 100$$ where,

I = the stomatal index

S = the number of stomata per unit area

E = the number of epidermal cells for the same area.

2.6. Chromosome Numbers

For the study of diploid chromosome numbers Tjio and Levan's (1950) oxyquinoline aceto-orcein squash method was followed.

Healthy root tips were cleaned and excised for

pretreatment in 0.002 mol. 8-Hydroxy-quinoline for 2 to 3 hrs. Root tips were then hydrolysed in a mixture of 2% orcein and 1 N HCl (9 : 1) by gently heating over a spirit lamp until the vapours appear. After cooling they were selected for squashing in 1% Aceto-orcein. Selected slides were sealed with gum-mastic, which remained good for observation even for a fortnight.

2.7. Seed and Seed Germination

Seeds obtained from the extensive collections of mature fruits of the plants growing in different study sites were cleaned and stored in glass-stoppered bottles at room conditions.

Seed size - Length, breadth etc. of 100 seeds were individually measured with the aid of Vernier callipers, and from these data mean length, breadth etc. along with their standard deviations were calculated.

Seed weight - Seeds were weighed in several lots of 100 seeds each, and from these data mean weight along with its standard deviation was calculated.

Moisture Content of Seeds - Freshly collected seeds were weighed in lots of 100 seeds each. Then they were kept in oven for drying at 60°C till constant weight, and moisture content was calculated from the loss in weight on drying.

Imbibition Rate - This was studied by keeping the seeds in separate lots of 100 seeds each in 6 ml of distilled water in a Petri dish covered with its lid. The imbibing seeds were

kept at room temperature, and increase in weight at regular intervals of time was recorded, and percentage of water imbibed was calculated.

Germination Experiments - These were carried out in Petri dishes (Corning - 9 cm diameter) keeping the seeds on a single filter paper (Whatman No. 1) kept moist with double glass distilled water. The Petri dishes were kept under laboratory conditions of temperature and light, unless stated otherwise. Emergence of radicle was taken as the criterion of germination. 50 weeds were taken in each Petri dish and 3 replicates were kept for each treatment. Each experiment was run for 20 days in case of Abutilon ramosum and Euphorbia geniculata, and for 30 days in case of Rivinia humilis. The number of seeds germinated was recorded once daily. The germinated seeds after being recorded were removed from the Petri dishes.

The seeds were surface-sterilized by treating them with 0.1% $HgCl_2$ solution for 1-3 minutes and then were thoroughly washed with distilled water before being kept for germination.

Percentage germination was calculated in all cases, and germination speed, viz., number of days required for 50 per cent of the ultimate percentage of germination was also recorded in quite a few cases.

The experimental data were statistically analysed, and are presented in tabular form and also graphically.

Details of other special methods or treatments for

germination experiments, wherever employed are mentioned in the text.

2.8. Culture Experiments

The experimental procedure has been given separately for each culture experiment along with the text.

2.9. Statistical Analysis

The statistical analysis of the experimental data was carried out using standard methods and procedures as given by Snedecor and Cockran (1967), Campbell (1967) and Parker (1973).

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