

## M A T E R I A L S   A N D   M E T H O D S

Plants for the present study were mostly collected from the states of Kerala and Tamil Nadu in South India situated between the north latitudes 8° and 20° and the east longitudes 74° and 86°. An index of localities of collection is provided in appendix I. The forest flora of the Western Ghats is rich in members of the Labiatae and most of the wild species reported in this study were collected from around the hill stations such as Ponmudi (450-900m), Courtallum (450m), Munnar (1050m), Devikolam (1200-1220m), Wynaad (1200m), Ootacamund (2040-2500m) and Kodaikanal (1800-2100m). Materials were also collected from other forest ranges in the Western Ghats like Ranni (500m), Muzhiyar (740-750m) and Silent Valley (1200m) in Kerala, Maruthamalai (432-435m) near Coimbatore and Maruthwamalai (369m) near Cape Comorin in Tamil Nadu and Nandi Hills (1100m) near Bangalore in Karnataka State. Some of the species were also collected from various places in Kerala such as Veli (40m), Trivandrum (50m), Vithura (106m), Mavelikara (85m), Changanacherry (80m), Calicut (45m), and Tellicherry (33m). A few exotic ornamentals obtained from the Public Gardens, Trivandrum, the Government Botanic Gardens, Ootacamund and the Lal Bagh Gardens, Bangalore were also studied. Most of the species collected were grown under greenhouse conditions in the Botanic Garden of the University of Kerala

Herbarium sheets of all species investigated were prepared and deposited in the Central Herbarium, Department of Botany, Kerala University (KUBOT). The accession numbers of the herbarium sheets are given in the observation part immediately after the names of the species. For the identification of plants Hooker (1885), Gamble (1924), Bailey (1960), Wight (1963), Graf (1978), Cramer (1981) and Rani and Mathew (1983) were referred. Species determinations were verified at the regional office of the Botanical Survey of India, Coimbatore and also by comparing with the original sheets of M. Rama Rao and J.S. Gamble in the Botany Department of University College, Trivandrum.

Chromosome counts were made primarily from PMCs and wherever available from root tip cells. For meiotic studies in the indigenous species flower buds were mostly fixed from plants growing in the wild state during field trips. But in cases where such flower buds failed to give PMCs at the correct stage of meiosis flower buds were fixed from transplants, grown in the garden. Root tips for somatic chromosome studies were obtained from seedlings or rooted stem cuttings grown in the Department Garden. Actively growing root tips were collected between 12.30 pm and 12.45 pm. Root tips were pretreated for 3 hrs in 0.002 M aqueous solution of hydroxyquinoline (Tjio and Levan, 1950). The small size of chromosomes in the plants and the

occurrence of essential oils in the tissues make the members of the family difficult cytological materials. Flower buds and root tips fixed in Semmen's fluid mixed in the ratio 3 absolute alcohol: 1 glacial acetic acid: 1 chloroform (Sharma and Sharma, 1965) were, however, found to give better results. One or two drops of ferric acetate was added to the fixative in order to enhance the stainability of chromosomes. Both meiotic and mitotic chromosomes were stained in 2 per cent aceto-carmin. Observations were made mostly from temporary paraffin sealed slides and photomicrographs were taken at a magnification of x 750. For preparing photographic plates the photographs were magnified to three times and then the plates were reduced to desired size. The chromosomes of majority of the Labiatae are small and in a few preparations some of the chromosomes were not sufficiently spread. Therefore explanatory diagrams of the preparations with the same figure numbers as in the plates are given intermittently in the observation part. For this, the chromosomes on enlarged photographs were outlined with lead pencil, bleached and filled with Indian ink. The figures were recopied photographically and reduced to desired magnifications.

Gross pollen sterility was determined by staining with aceto-carmin. Mature anthers were smeared in a 1:1 mixture of 1 per cent aceto-carmin and glycerine. The slides were kept for 30 minutes before counting. Pollen sterility was estimated by scoring the unstained pollen grains as sterile. Percentage of

sterility was assessed based on a sample of about 1000 pollen grains in each species obtained from counts of three slides.

For the arrangement of tribes and genera the classification of Bentham (1876) is followed along with the corrections in the names of the tribes and subtribes (Sanders and Cantino, 1984).