CHAPTER 3: MATERIALS AND METHODS

Present cytomorphological investigations are carried out on wild members of family Poaceae growing in different localities of study area i.e. states of Punjab, Haryana, Rajasthan, Himachal Pradesh and Madhya Pradesh. The study includes floristic surveys, meiotic studies and preparation of database on chromosomal diversity of 128 species of the family Poaceae.

3.1 Floristic surveys:

For the collection of materials during present study, a total of 28 survey trips have been made to various localities of North-West and Central India, throughout the year from 2006-2011. Four specimens of each species were collected, wrapped in blotting sheets and then subjected to pressing and dried up for preservation. As a result of these surveys, a total of 577 populations/accessions have been collected. The information regarding distribution pattern, morphology, phenology, etc. of collected plants was gathered. The taxa having wider distributional range were collected on accession basis from varied altitudes. Field photographs of the few specimens were captured for further help in the identification of the species. Particulars of the studied areas are shown in maps (Plates A-D). Photographs of various vegetation patterns along with different forest types have been captured (Plates I-IV).

For the present investigations, cytological survey of grasses has been carried out from different regions of North India (Himachal Pradesh, particularly Lahaul-Spiti), North-Western India (Punjab, Haryana & Rajasthan) and Central India (in Satpura range of Madhya Pradesh). The exact locations of these regions are indicated in maps (Plates A-D). The areas surveyed from different states are shown shaded in the maps with their exact locality.

3.2 Fixation:

Young inflorescences were collected during day time (8am – 4pm) and fixed in Carnoy’s fixative (6 parts absolute alcohol: 3 parts chloroform: 1part glacial acetic acid v/v/v) for 24 hours and then transferred to 70% alcohol for preservation at 4°C.
3.3 Identification of Plant specimens:

Identification of plant specimens have been done by consulting various floras, namely, Flora of British India, Flora of Lahaul-Spiti (Aswal & Mehrorta 1994), Flora of Kullu (Dhaliwal & Sharma 1999), Flora of Himachal Pradesh (Chowdhery & Wadhwa 1984), Flora of Patiala (Sharma & Bir 1978), Grasses of Punjab plains (Nair 1978), Flora of Rajasthan (Sharma & Tiagi 1979), Roy (1984), Shetty and Pandey (1983), Shetty and Singh (1987, 1991, 1993), Bhandari (1990), Prasad et al. (1996) and Sharma (2002). Further confirmation of these specimens has been done from Herbaria, Punjabi university (Patiala), Forest Research Institute, and Botanical Survey of India (Dehra Dun) and Panjab University (Chandigarh). The identified plant specimens have been deposited in herbarium (PUN), Punjabi University, Patiala and PUN numbers of authenticated accession have been obtained.

3.4 Meiotic Studies:

The identified species subjected to detailed meiotic analysis including meiotic chromosome numbers, meiotic course, microsporogenesis, pollen fertility percentage and pollen grain size. Smears of pollen mother cells (PMCs) are prepared in 1% acetocarmine (Prepared by fluxing Carmine in 45% acetic acid). Pollen fertility is estimated by crushing the mature buds in glycerol-acetocarmine (1:1) and kept for 24 hours. Well stained pollen grains were considered as fertile and shrivelled or unstained nuclei as sterile. Measurement of pollen grain size is taken using ocular micrometer. Chromosome numbers were determined at Diakinesis, Metaphase-I (M-I) and Anaphase-I (A-I) from freshly prepared slides using compound light microscope. Large numbers of pollen mother cells were observed at all stages of meiosis for determination of meiotic behaviour. For stomatal studies, mature leaves were treated with KOH for 10-20 minutes (depends upon the leaf texture), and the epidermal layer peeled off is stained with safranin.

3.5 Photomicrography:

Photomicrographs of chromosome counts were made from freshly prepared slides using Leica Qwin and Nikon 80i digital imaging systems.
3.6 Morphometric analysis:

To check any morphological variation at intraspecific level, the related accessions were subjected to detailed morphological (micro- & macro-characters) evaluation on qualitative and quantitative basis. For stomatal studies, mature leaves were treated with KOH for 10-20 minutes (depending upon the leaf texture), and the epidermal layer peeled off is stained with safranin.

3.7 Compilation of chromosomal data:

NORTH INDIA:
- Himachal Pradesh

NORTH-WEST INDIA:
- Punjab
- Haryana
- Rajasthan

CENTRAL INDIA:
- Madhya Pradesh
MAPS OF (I) RAJASTHAN, (II) HARYANA & (III) PUNJAB

PLATE-C
MAP OF MADHYA PRADESH

PACHMARHI
- Dhupgarh (1200m)
- Mahadev (550m)
- Dokri Khera (1150m)
- Pandu Caves (1050m)
- Cantonment Area (1050m)
- Jatta Shankar (750m)
- Big Fall (900m)
- City Garden (1150m)
Views of Spiti Valley

Key Monastery
Kunzum pass, 4551 m

Salix forest near Chandra River

Kibber Village, 4270 m

A view of Agricultural field
View of Pachmarhi

A view of Park at Pachmarhi

Pandu caves

A view of vegetation

Handikho

Dhupgarh

Big Fall
VIEWS OF NORTH -WEST INDIA

Mount Abu (Rajasthan)

Guru Sikhar (Rajasthan)

Wheat field in Punjab

Plains of Haryana