CHAPTER-IV:
METHODOLOGY
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a. Literature survey:

General information on grasses including lithophytic ones of Maharashtra and surrounding areas with respect to their occurrence, distribution, taxonomy, status, diagnostic features, ecology and cytology was gathered through consultation of national and regional floras, books and research publications. Similarly data on rock types, climate, geology and drainage-patterns was obtained by referring Gazetteer of Maharashtra and relevents books (Washington, 1922; Deshpande, 1971; Dikshit, 1986; Deshpande, 1998; and Jaymala et.al. 2002) and various research publications.

b. Collection of specimen and herbarium preparation:

The work on collection and documentation of lithophytic grasses was initiated in 2007. During last four years about 80 field trips of short and long duration (1–6 days) were undertaken for extensive and intensive survey and collection of lithophytic grasses from Maharashtra state. Over 800 specimens were collected from selected localities of Maharashtra during last 4 years.

Frequent visits were made to special habitats to collect lithophytic grasses and to study their adaptations. During study period, as far as possible, the important localities of rocky substratum in Maharashtra have been covered. The important field notes were written on the spot and field numbers were given to each grass specimen. Also the, grasses with roots were collected and grown in Botanic garden, Department of Botany, Shivaji University to study morphology, cytology as well as adaptative features if any.

By using regular drying method collected grasses were properly processed with blotting papers and newspapers following instruction by Jain (1977). After proper processing and poisoning, the specimens were mounted on herbarium sheets and deposited in the Herbarium of Botany Department, Shivaji University, Kolhapur after confirmation of their identity. The identifications were confirmed by comparing the specimens with authentic specimens in SUK and BSI herbarium.

c. Morphology:

Most of the grasses collected during last four years have been described and photographed. Spikelets were dissected under Labomed Zoomer Sterezoom
microscope and dissected parts were measured under loop scale. Photographs of spikelets were taken under Carl Zeiss Microscope and Nikon SMZ 800 Stereozoom with MDC lens using Coolpix 4500 and 8400 digital camera.

Most of the grasses have been identified satisfactorily by means of available literature. The important floras used in determination are ‘Grasses of Marathwada’ (Patunkar, 1980), ‘Grasses of Maharashtra’ (Deshpande and Singh, 1986), Flora of Marathwada (Naik, 1998), ‘The Bombay Grasses’ (Blatt and McCann, 1935), ‘Flora of Maharashtra State–Monocotyledones’ (Sharma et. al., 1996), ‘The Grasses and Bamboos of India’ (Moulik, 1997), ‘Grasses of Burma, Ceylon, India and Pakistan’ (Bor, 1960), ‘Flora of Kerala Grasses’ (Sreekumar and Nair, 1991), ‘Genera Graminum’ (Clayton and Renvoize, 1989) and ‘The Grass Genera of the World’ (Watson and Dallwitz, 1994). Identifications of doubtful specimens were confirmed in BSI herbarium, Poona. Some few grasses, which could not be assigned to any Indian species, have been sent to experts for their comment on identity of the specimens. During our studies, Dr. J. F. Veldkamp, National Herbarium, Netherlands; Prof. Hildemar Scholz, Freie Universitaet Berlin, Dr. T. A. Cope Royal Botanic Gardens, Kew and Neil Snow, Botanist, Herbarium Pacificum (BISH), Bishop Museum, U.S.A. helped in confirmation of identification of some grasses and latin diagnosis for novelties in grasses.

Classification of grasses by Bor (1960) has been adopted for presentation of the data. The keys have been provided for easy identification for all the tribes, genera, species and varieties of lithophytic grasses. These keys are artificial and strictly dichotomous. The arrangement of genera, species and interaspecific categories has been made sub-family and tribe wise. Nomenclature has been updated with latest taxonomic literature available. Detailed description based on observations have been prepared for each taxon. Description is followed by ecological note on phenology, distribution, a critical note if any on identity, variation and nomenclature.

Detailed studies on cytology, caryopses morphology and seed germination was made by using standard methodology.

d. Cytology:

Flage leaf inflorescences with spikelets in bud condition were fixed in modified Conroy’s fixative (Ethanol and Glacial Acetic Acid 3:1) in mornning
time *i.e.* between 7.00 to 8.30 am. Smear preparations were made from young anthers. Anthers were smeared in propionic-orcein and analyzed for meiotic chromosome counts. Photographs were taken with CARL ZEISS JENAVAL compound microscope.

**e. Caryopsis morphology and seed germination:**

Morphology of seed and embryo was studied by soaking seeds for more than 12 hours in distilled water. Photographs were taken with Nikon SMZ Stereomicroscope. Seeds were germinated in petriplates and careful observations were made on emergence of coleoptile and radical in some of the lithophytic grasses.

**f. Field observation:**

Adaptations in different grasses were carefully studied and noted through intensive field visits to various localities with different kind of rocks. Seed germination done in laboratory under controlled climatic condition. The morphological peculiarities in roots, stems and spikelets were tried to correlate with ecological nitches in which the grass grow.

Careful field notes were prepared in the field in relation to various rocky substratum and grass community of the locality. Inventory of grasses on different rocky substratum *viz.* laterite, lateritic plateaus, ignitious rocks, murum, hanging rocks, steep rocky substratum etc. was studied. Grass species associates in different ecological nitches and their adaptive features were studied and carefully analysed.

The root system, spikelets and associated structures such as, callus, awns, ornamentation on glumes etc. were studied carefully to understand their adaptive significance and relation with habitat in which they grow.

Careful observations were made on distributional pattern of grasses with respect to altitude, slopes and various kinds of rocky substratum found in Maharashtra. Attempts were made to correlate the morphological peculiarities of grasses with environmental factors to understand their signification in distribution and diversification of grasses in the region.

Special efforts have been made to study and understand the diversification and speciation of the endemic genus *Glyphochloa* in Western Ghats and to through the light on its biosystematic aspects.