CHAPTER IX

MATERIAL AND METHODS
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Present study deals with some ecological aspects of *Themeda quadrivalvis*, one of the dominant species in grasslands of Sagar. General methods which are followed in this study are given in this chapter.

Sites:

A complete survey of local grasslands was done before the selection of site. *Themeda quadrivalvis* populations were found to occur on slight hill slopes, also at foot hills. On hill tops it was found to be mixed with *Heteropogon contortus*, *Cymbopogon martinii* and *Iseilima laxum*. For studying phytosociology and environmental factors an area of 10 mx 10m *Themeda quadrivalvis* grassland was protected by fencing. For the study of intraspecific population variations three sites were selected, namely, *Makronia*, *Patharia* and *University campus*. These sites varied chiefly in soil characters. For seed germination and reproductive characters (seed output and reproductive capacity) studies, samples were collected from a large number of localities and habitats (Table-5).

Germination studies:

Seeds collected from various localities were brought to laboratory in moisture proof containers and stored for six months. TTC test for viability was performed before germination studies. Germination experiments were done in Petridishes containing moist cotton and filter paper. Effects of different light exposures, chemical treatments and growth regulators were
studied on germination. These observations were recorded for 15 days and results were recorded as percentage germination in treatment concerned. Effects of various types of soils and calcium contents were also studied in pot experiments. Details of the procedures followed for individual treatment are given in Chapter 10.

Growth studies:

In present investigation growth studies were conducted both for seedlings and for plants. For seedlings the experiments were done in Petri dishes and growth of plumule and radicle was recorded up to 7 days after germination. Effects of various chemicals and growth regulators were studied on seedling growth Chapter 11.

To study the growth behaviour of plant, experiments were conducted in earthenware pots. Seeds were sown in July and watered every alternate day. After 15 days of emergence of plants, a spray of growth regulators (IAA, IBA and GA) was made in different concentrations (1 to 50 ppm). The spraying was done thrice a month, in August, September and October. After complete flowering and fruiting plants were dug out from the pots and root and shoots dry weights were recorded. During these three months of growth observations for morphological characters were noted to determine growth rates in different plant parts. All growth studies, control as well as treatments, were done in triplicate. All growth observations, treatments etc. are given in Chapter 11.

Phytosociological studies:

These studies are based on the observations taken in fenced *Themeda quadrivalvis* grassland in the University Campus.
To get complete view of the grassland vegetation phytosociological characters were studied in August, September and October. These three months constituted complete growth period and thus changes in structure and composition in the vegetation that might occur during this active period, are considered in present study.

Sampling was done generally in the first week of the month. The data for a month is based upon 10 quadrats of 1 m x 1 m size. The analytical characters like relative density, relative frequency, relative abundance and importance value index were calculated with the help of following formulae.

Relative density = \( \frac{\text{Number of individuals of the species}}{\text{Number of individuals of all species}} \times 100 \)

Relative frequency = \( \frac{\text{Number of occurrences of species}}{\text{Number of occurrences of all species}} \times 100 \)

Relative abundance = \( \frac{\text{Total number of individuals in all the quadrats studied}}{\text{Total no. of quadrats of occurrence}} \times 100 \)

Importance value index = RD + RF + RA (Mishra & Jain 1972).

Intraspecific variations:

To study intraspecific variations with \textit{Themeda quadrivalvis} populations, samples were collected from University campus, Makronia and Patharia. Sampling was done in flowering and fruiting seasons. These variation studies are based upon 14 morphological characters. This large number of characters was taken to measure maximum morphological variations that may occur in the populations. Observations for these characters are based on intense sampling and are mean of 1000 individual measurements. Multivariate analysis was done for statistical comparisons of these characters between the samples of different localities.
Soil and Plant analysis:

(A) Soil analysis:

Soil was collected from different localities of Themeda quadrivalvis from a depth of 10 cm to 30 cm depending upon the soil depth. All these samples were mixed together to get composite samples, packed in polythene bags and were brought to laboratory. In these samples moisture content pH value, Organic carbon and nitrogen, total sesquioxides were determined by standard methods given by Piper, (1944) Exchangeable bases were determined flame photometrically (Jackson, 1962). Calcium carbonate was determined by Collin's calcimeter.

(B) Plant analysis:

Plant analysis was done to determine protein and fat contents in control and in plants treated with growth regulators. These studies were made in the month of September, before initiation of flowering and the plants were in maximum palatable condition. The main object of this analysis was to see the effects of growth regulators on protein and fat content of the plant material and to acess its comparative fodder value.

Fat contents were determined by petroleum ether extraction method and for proteins method given by Morrison and Pirie (1961). In this method the samples were kept in alkaline medium for two days and after it the medium was changed to acidic side. This solution was heated on 70 - 30°C. Thus the coagulated proteins were filtered out.

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