CHAPTER III

MATERIALS AND METHODS
The first part of this chapter deals with the materials and methods used in Chapter 4 on seed germination studies, whereas the second part deals with the methods used in Chapter 5 on seedling growth.

**Part I**

**Collection of seeds/fruits**

The propagules of trees in tropical deciduous forests are found lying over the forest floor either in dehiscent or indehiscent forms. In trees like *A. catechu*, *L. parviflora* and *T. populnea*, the dehiscence of fruits take place and the seeds fall down on the ground. On the other hand in tree species like *T. grandis*, *T. tomentosa* and *T. bellerica* the seeds remain in the locule of hard woody fruit and are dispersed as such. Hence in the present study, term 'Seed/fruit' has been used throughout in germination and seedling experiments.

 Seeds/fruits of *A. catechu*, *A. latifolia*, *D. melanoxylon*, *L. parviflora*, *T. grandis* and *T. tomentosa* were collected from medium sized parent trees, occurring in local forests (Bandri - on Sagar-Jhansi road, 28 Km from Sagar, Jaruakhera on Sagar-Bina road 38 Km from Sagar). Atleast ten individuals of parent trees of each species were selected for collection of seed/fruit to get a composite sample with a view to
include different seed types. Besides, the seeds of other species were obtained from Forest Department Sagar. Fruits of T. grandis were also supplied by Conservator of forests, Palode, Kerala representing the seed population of South India. Composite seeds/fruits of each species after cleaning and drying were stored in polythene bags at room temperature. Following forest tree species have been taken for different experiments during the study: Acacia catechu, Acacia auriculaeformis, Albizia procera, Anogeissus latifolia, Anogeissus pendula, Bauhinia retusa, Butea monosperma, Cassia fistula, Cieba pentandra, Dendrocalamus strictus, Diospyros melanoxylon, Dalbergia sissoo, Emblica officinalis, Lagerstroemia parviflora, Mitragyna parvifolia, Ogeinia dalbergioides, Fongamia pinnata, Teckona grandis (Local and southern variety), Terminalia bellerica, Terminalia tomentosa, Thespesia populnea.

Treatment of seed/fruit

In all the species except T. grandis (local variety) seeds/fruits were used without any specific treatment. In case of T. grandis (local variety), fruits were treated with cow dung and lime (3:1) and kept moist by watering to soften the outer hard woody fruit wall.

Three replicates of 50 seeds/fruits of each species were used in all the experiments. The sound seeds/fruits
(selected by floatation test) were surface sterilized with
0.1% HgCl₂ for five minutes and then washed with sterilized
distilled water five times. The emergence of radicle above
the surface soil and potting media or the appearance of radicle
on germination paper was taken as the main criterion for the
germination. The observations were made daily for 30 days
with watering being done on alternate days. The germinated
seeds/fruits after counting were removed to minimize the
difficulties in counting of freshly germinated seeds/fruits.

**Nursery**

The seeds were uniformly sown (0.5 to 1.5 cm depth
depending upon the size of seeds/fruits) in 1 x 1 meter
area of nursery beds in Government Forest Nursery near
Ranipura village, about 12 Km from Sagar city on Sagar-Jhansi
road.

**Potting media**

Black soil, sand and one month old saw dust (Bollen, 1969) were used to prepare following three media:
I - 100% soil, II - 50% soil and 50% sand, and III - 50% soil
and 50% saw dust. Fifteen earthen pots of medium size were
used for each media and 10 seeds/fruits were sown per pot
at the depth of 0.5 to 1.5 cm.
Litter

The composite litter was collected from nearby Patharia forest. Three litter depths 0-1, 2-3 and 4-5 cm were made above the soil surface in medium size earthen pots. Ten seeds/fruits of each species were sown in between soil and litter surfaces. In control, no litter was used above the soil surface. Fifteen replicates per depth were used.

Laboratory

One hundred fifty seeds/fruits were placed on the tray covered with sterilized and moistened germination paper (supplied by F.R.I. Dehra Dun as per I.S.T.A. standard) in a germinator at 27±2°C and 90% humidity.

Imbibition

The seeds were imbibed in glass beaker containing sterilized water for 3, 6, 9, 12, 24 and 48 hours in an incubator at 27±2°C. The imbibed seeds were then germinated on sterilized and moistened germination paper in seed germinator at 27±2°C and 90% humidity.

Dimension

The seeds/fruits of each species were segregated into three seed classes on the basis of their weight. Ten seeds of each weight class were then sown in 15 earthen pots.
Leachate

Ten g and 25 g pure litter of *T. grandis*, *T. tomentosa*, *L. camara*, and *B. monosperma* (collected from nearby Patharia forest) were soaked in 100 ml distilled water for 24 hours and 120 hours. To study the effect of these four types of leachates, the seeds of *T. grandis* were placed in sterilized petri dishes, lined with layer of filter paper which was moistened with 10 ml of leachate. The petri dishes containing the seeds were then incubated in a seed germinator at 27±2°C and 90% humidity.

Part - II

Seedling growth

Choice of species

The following species were selected to study the growth of seedlings: *G. fistula*, *D. sissoo*, *O. dalbergioides*, *T. grandis* and *T. tomentosa*. These species were selected because these are either dominant or codominants in dry deciduous forests of the study area, and are commonly used for afforestation and reforestation practices. Moreover, their seeds are easily available.

One month old seedlings of equal size of *G. fistula* *O. dalbergioides*, *T. grandis* and *T. tomentosa* were transplanted at 10 x 10 cm spacing (to avoid effect of shading) in open plots (10 x 1 m) containing black soil in
Botanical garden, University of Sagar, Sagar (23° 50' N latitude and 78° 40' E longitude). Watering was done on alternate days. A careful digging was done for uprooting the seedling. Seedlings were taken every month from August 1980 upto July 1981 at an interval of thirty days from the date of planting. Soil was removed from the seedlings by repeated washing then seedlings were separated into root/shoot fractions for fresh weight and leaf area determinations. Dry weight of each fraction was taken after drying in oven for 48 to 72 hours at 80°C. Five replicates of seedlings were used for each species.

**Growth analysis**

From leaf area (A) and dry weight (W) data, following calculations were made according to Okali (1970):

\[ \text{Mean leaf area ratio (F)} \, \text{cm}^2 \cdot \text{g}^{-1} = 1/2 \left( \frac{A_1}{W_1} + \frac{A_2}{W_2} \right) \]

\[ \text{Net assimilation rate (NAR)} \, \text{g} \cdot \text{m}^{-2} \cdot \text{week}^{-1} = \left( \frac{W_2 - W_1}{A_2 - A_1} \right) \ln \left( \frac{A_2/A_1}{t_2-t_1} \right) \]

\[ \text{Relative growth rate (R)} = \text{mg} \cdot \text{g}^{-1} \cdot \text{week}^{-1} = \ln \left( \frac{W_2/W_1}{t_2-t_1} \right) \]

Where \( t_1 \) and \( t_2 \) represent the time of first and second harvest respectively. In represents neopararian log.
The effects of different combinations of soil, sand and saw dust as different potting media were studied on T. grandis, O. dalbergioides, D. sissoo. Thirteen potting media were prepared by mixing black soil, sand and saw dust in different proportion by volume (Table 2). Sand and saw dust were used after sieving separately through I.S.S. mesh number 200. Each medium was filled in fifteen polythene bags. The seedlings of equal size were transplanted in these media. Sufficient watering was done on alternate days. After six months, seedlings were removed from polythene bags with intact root system for measurements of fresh and dry weights (g) moisture content, length (cm), leaf area (sq cm), number of secondary roots. Eight seedlings were taken for these measurements.

The characteristics of above and below ground parts was assessed for some important forest tree seedlings (including species of 1st experiment) and also in some fast growing species which are widely used in large scale plantations e.g. A. catechu, A. auriculaeformis, A. latifolia, A. pendula, B. retusa, G. fistula, G. pentandra, D. melanoxylon, D. sissoo, E. officinalis, I. parviflora, M. parvifolia, P. pinnata, T. beleica, T. grandis(s), T. grandis, T. populnea and T. tomentosa.
Table 2: Different potting media used during seed germination and seedling growth studies.

(Sl = Soil; Sd = Sand; St = Saw dust; 0, 1, 2, 3 and 4 represent 0%, 25%, 50%, 75% and 100% volume/medium respectively)

<table>
<thead>
<tr>
<th>Potting media number</th>
<th>Composition of media</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sl₀ St₀ Sd₄</td>
</tr>
<tr>
<td>2</td>
<td>Sl₀ St₂ Sd₂</td>
</tr>
<tr>
<td>3</td>
<td>Sl₀ St₃ Sd₁</td>
</tr>
<tr>
<td>4</td>
<td>Sl₀ St₄ Sd₀</td>
</tr>
<tr>
<td>5</td>
<td>Sl₁ St₁ Sd₂</td>
</tr>
<tr>
<td>6</td>
<td>Sl₁ St₂ Sd₁</td>
</tr>
<tr>
<td>7</td>
<td>Sl₁ St₃ Sd₀</td>
</tr>
<tr>
<td>8</td>
<td>Sl₂ St₀ Sd₂</td>
</tr>
<tr>
<td>9</td>
<td>Sl₂ St₁ Sd₁</td>
</tr>
<tr>
<td>10</td>
<td>Sl₂ St₂ Sd₀</td>
</tr>
<tr>
<td>11</td>
<td>Sl₃ St₀ Sd₁</td>
</tr>
<tr>
<td>12</td>
<td>Sl₃ St₁ Sd₀</td>
</tr>
<tr>
<td>13</td>
<td>Sl₄ St₀ Sd₀</td>
</tr>
</tbody>
</table>
The seeds of each species were sown at 10 x 10 cm spacing (0.5 to 1.5 cm depth) in 10 open plots (10 x 1 M) containing black soil in State Government Forest Nursery Ranipura on 14 July 1981. Watering was usually done on alternate days. The length of seedlings was measured after 3, 9, and 12 months.

After one year, five seedlings of each species were carefully digged out for the measurements of leaf, shoot, root fresh and dry weight and moisture content. In addition, the root collar circumference, length of tops, length of primary and secondary roots, number and circumference of secondary root, length of that portion of primary root which has maximum number of secondary root, were measured.