CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

The Siwalik hill ranges extend from Jammu to West Bengal and beyond in a continuous chain in front of Himalayan mountain system. They are geologically recent in origin and are composed of semi and unconsolidated deposits prone to rapid erosion. They are present in three districts of Haryana state (core area of investigation). Owing to anthropogenic causes the problem of habitat degradation has aggravated. Some of the economically important indigenous trees are not regenerating to required extent. It has become necessary to investigate the causes of absence or inadequate regeneration and suggest remedial measures. For this purpose four species were selected as under, *Terminalia chebula* Retz., *Terminalia arjuna* Wright & Arn., *Holoptelea integrifolia* Planch., and *Artocarpus lakoocha* Rox.

Modern agriculture and cattle rearing have not benefited inhabitants Siwalik hills to the same extent as in fertile plains with assured irrigation. Watersheds of Satluj (Sarsa) Ghaggar, Tangri, Markanda and Yamuna rivers, are getting adversely affected. Livelihood security of agricultural plains is inextricably related to conservation of soil, water and rich biodiversity of plant species in hills. A protective cover of economically important indigenous trees in hills is very essential for ensuring conservation. At present there is limited Government effort in carrying out forest plantations in hills. That should be associated with private effort of raising trees under integrated food and tree cropping systems. By adoption of this approach in the long run qualitative improvement of habitat would be possible.

In northern districts of the Haryana state intensive agriculture is associated with equally intensive farm and agroforestry operations. Peasants have come out of vicious circle of poverty by practicing combination cropping of food crops and timber yielding
trees viz., *Populus deltoides* Gertn. and *Eucalyptus tereticornis* Smith. These two exotic species are compatible with wheat-paddy, sugarcane and fodder crops as they are winter deciduous and light-crowned respectively. That combination has not worked in the hills as both species require irrigated deep soils which cannot always be provided in hills consequently they have lower rate of growth. Some of the trees studied have the potential for combination cropping in hills hence the investigations for genetic improvement of planting stock for improving productivity.

### 3.1.1 Climatic conditions

Details of three major components of climate viz., temperature, rainfall and relative humidity that generally prevailed in the area where most of the experimental trials were carried out is given in Figure: 3.1. This information was obtained from meteorological observatory (nearest) of the Central Soil Conservation Research Institute, Research Station at Manimajra, Union Territory of Chandigarh.

### 3.2 Chebulic myrobolam (*Terminalia chebula* Retz.) harar

#### 3.2.1 Introduction

Present investigations were undertaken with the plant species *Terminalia chebula* Retz. Fruits of this species in commerce are referred to as ‘chebulic myrobolam’ It occurs naturally in the Siwalik hill ranges of Haryana state. It has been described in the Forest Flora for the Punjab with Hazara and Delhi and Flora of Haryana (Parker, 1950; Kumar, 2000). It is a long-lived, hardy, deciduous tree that grows in hilly areas and its cultivation can be extended to adjacent well-drained plains. Fruits are valued for tanning leather, dyeing textiles and preparation of many traditional Indian medicines of *Ayurveda* (Sanskrit =science of life) system. It is reputed to be one of the six most important ‘*materia medica*’ of *Ayurveda*. It is a multi-use tree as wood can be used as timber, for furniture manufacture and as firewood. During fodder scarcity leaves get used as cattle feed. Regeneration of this species in rapidly deteriorating habitat of Siwalik hill ranges is virtually non-existent as fruit / nut / seed is extracted raw (premature) for making ‘*murabba*’ and conditions for re-vegetation are negligible.

### 3.2.1a Following major areas were studied in case of ‘harar’:
trees viz., *Populus deltoides* Gertn. and *Eucalyptus tereticornis* Smith. These two exotic species are compatible with wheat-paddy, sugarcane and fodder crops as they are winter deciduous and light crowned respectively. That combination has not worked in the hills as both species require irrigated deep soils which cannot always be provided in hills consequently they have lower rate of growth. Some of the trees studied have the potential for combination cropping in hills hence the investigations for genetic improvement of planting stock for improving productivity. See table 4.27a & Figure 4.6a

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3.2.1a. Following major areas were studied in case of ‘harar’:
• Survey of base population in three provenances located in the Siwalik hill ranges of Haryana state. Identification of healthy phenotypic superior trees that bear large size fruits in greater quantity, called as candidate plus trees (CPTs).
• Tree-wise (CPT-wise) collection of raw and ripe fruits during different stages of maturity that were used for germination studies.
• Raised tree-wise (CPT-wise) seedlings for carrying out plantations.
• Carried out clonal propagation (vegetative), of juvenile and mature stem cuttings, especially those obtained from CPTs.
• Through conventional horticultural method of bench grafting, initiated multi-location (three locations) germplasm collection currently used as budwood source to be developed further as a grafted seedling seed orchards (GSSO).

3.2.2 Details of survey of base population

The base population was surveyed in Morni hills of Panchkula district. Darpur and Kalesar forests of Yamuna Nagar district of the Haryana state; Kothi village of Bilaspur district; Philorhi village of Nahan District of the Himachal Pradesh state and man made plantations of sectors 16 and 31-D of Chandigarh, Union Territory. Details of district and geographic location are given in Table: 3.1.

3.2.2. a. Method of selection of CPTs

Following methods were adopted for collection of information regarding base population. They were surveyed during three successive fruiting seasons of 2000-2002.

i. Reconnaissance survey followed by total enumeration (measurement of girth over bark both at collar and 1.37 meters height and numbering) followed by visits of natural and artificial populations of species before fruit picking during October;

ii. collection of information from traditional ‘harar’ traders;

iii. consulted other workers engaged in T. chebula, Retz. tree improvement;

iv. consulted literature; and

v. collected and measured of size and weight of fruits of the tree.

Those healthy trees that bear exceptionally large size fruits in greater quantity (yield / tree) were selected as ‘candidate plus trees’ (CPTs). They were arranged in decreasing order of magnitude based on weighted criterion method on average index score as used by Institute of Forest Genetics and Tree Breeding, Coimbatore. Selection intensity was one out of 200 trees i.e., 0.55 %. Different characters that were studied for selection of
Figure 3.1

Rainfall in millimeters recorded at Manimajra Soil Conservation Research Center of ICAR, GOI, the nearest meteorological observatory to Panchkula.

Monthly Mean Minimum Temperature °C

Monthly Mean Maximum Temperature °C

Rainfall in millimeters

Mean Humidity% 7.00 am

Mean Humidity% 2.00 pm

25
CPTs are as under. The basic criterion of selection of CPTs has been economic expression of biological traits of fruits in terms of annual value addition or 'earning capacity' of selected trees.
Table 3.1 Details of natural populations of *Terminalia chebula* Retz. in Siwalik hills and avenue and grove plantations in Chandigarh

<table>
<thead>
<tr>
<th>Name of the Provenance</th>
<th>District</th>
<th>Geographic Location N.Latitude  E. Longitude</th>
<th>Base Population (No.)</th>
<th>CPTs (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Brahmpura-Philorhi</td>
<td>Yamuna Nagar</td>
<td>30° 25’’ 77° 10’’</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>ii. Salyo-Hathia</td>
<td>Panchkula</td>
<td>30° 41’’ 77° 10’’</td>
<td>2350</td>
<td>7</td>
</tr>
<tr>
<td>iii. Mandhna,</td>
<td>Panchkula</td>
<td>30° 35’’ 77° 05’’</td>
<td>180</td>
<td>3</td>
</tr>
<tr>
<td>iv. Chandigarh</td>
<td>Chandigarh U.T</td>
<td>30° 35’’ 77° 05’’</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>2646</strong></td>
<td><strong>14</strong></td>
</tr>
</tbody>
</table>

Selection intensity: Number of CPTs selected for every 1000 trees surveyed. In this case 14 CPTs were selected for 1000 trees surveyed; expressed as percentage 0.55%.
The gradation list of CPTs based on weighted criterion is explained for each trait as under.

3.2.2. b Fruit characters

i) Yield: Annual yield of fruits by weight (raw-fresh) expressed as function of basal area of a tree at collar level measured in square meters is considered as a reference for comparison. As the CPTs belong to different ages, unless a datum is introduced comparison becomes unreasonable, therefore, the method. Mean value of fruit production for wild trees is 350 kg / Sq. m (three to four trees per one Sq. m of basal area) of basal area. Any increase in 10 kg over mean value of base population was given an index mark of 1.

Example CPT No.1 Produced 450 Kg of fruits per Sq. m of basal area; subtract the base population mean of 350 Kg; the difference is 100; resultant is divided by 10; therefore, index marks are 10. Since annual yield is the most important character greater weight was given to that trait.

ii) Consistency of Annual Production: Consistency in bearing known quantity of fruits from year to year of a CPT is of considerable importance particularly because fruit collection rights are sold much in advance. How often there has been regular bearing during last six years was recorded and each year’s bearing allotted 0.5 marks and compared with the wild variety.

iii) Mean weight: Variation in mean weight of fruits exists within and between the trees. This is resultant of series of blooming episodes and also how many fruits develop within a bunch. Within the same tree, in a fruit bearing bunch, if number of fruits are smaller, their size is larger and vice versa. Larger fruits are borne on terminal locations of a bunch and smaller fruits on sub-terminal locations. For commercial purposes fruits belong to three categories as explained earlier, dried infant fruits, raw fruits and dried mature fruits. Mean value for infant fruits was not taken as size difference is much within the same tree as different stages of maturity are mixed and drying is not done tree-wise, other two commercial categories were given index score as explained under.

iii) a. Green fruits vary between 15 g to 35 g in weight and commercial grading into five categories is done by traditional graders and range of grade was generally five grams, i.e., 35; 30; 25; 20 and 15. Out of the five grades, first to fourth grade were classed as ‘murabbi’ i.e., superior and fourth to fifth grade as ‘kachri’ i.e., inferior. Depending upon demand 20 gram fruit can be classified as murabbi or kachri i.e., either medium quality or
low quality. Incremental premium is there for every higher grade, and indexing is done as follows.

Weight in grams = 35; 30; 25; 20; 15
Index marks = 8; 4; 2; 1; 0.

iii) b. Only one index score for raw fruits has been given for convenience as giving index score to dry fruits leads to double counting as fruits are sold either raw or dry.

iv). Mean volume: At the time of commercial grading volume is one of the criteria, five commercial grades are made on volume basis for pricing raw fruits. As index score has already been given for related character viz., fruit weight except in varieties that make significant difference index score has not been given.

v). Nut to pulp ratio: Ratio of the gravimetric quantity of pulp to nut measured in grams.

vi). Shelf life: Shelf life of raw fruits is counted in days and those fruits with more water content deteriorate rapidly.

vii). Fruit drop: Right from the time of fruit setting (May) up to shedding of ripe fruits (next April-May) continuous fruit drop is experienced. A scale of low, moderate and high each with a value of 1, -1 and -2 for every 10 kg of fruits dropped prematurely was assigned.

viii). Fruit and seed infestation: Damping off of raw fruits is a major cause of loss to dealers, presence of borers within the seed and mesocarp of some trees is a known disadvantage hence of negative selection worth.

ix). Colour of the fruit: Those fruits with yellow colour or ‘red blush’ are preferred to other fruits and colour is of positive selection value.

3.2.2. c Tree characters

x). General health of the tree and longevity: General health and vigour of the tree had a very important bearing on fruit production. As it was a long lived tree fungal / insect resistance of wood was very important trait that needs to be considered. Major limbs snap owing to hollow interior when the tree advances in age beyond 60 years which was the major reason of elimination of old trees. Trees that have survived for more than 80 years have something in them that had given them the stamina for survival. Such trees have positive selection value and should be preferred.

xi) Site advantages: Site on which a tree is situated has a very important role even while the genotype is superior. For the sake of comparison weight was either increased or
decreased depending on situation of the tree either in favourable or unfavourable location.

xii) Reputation for medicinal properties: Some trees had established reputation for being more effective for medicinal use. As proven source of medicine they command better price.

3.2.3 Seed germination studies

3.2.3.1 Introduction

Investigations were carried out to understand the reasons responsible for inadequate / scanty natural regeneration and one of the steps was seed germination studies. To determine factors affecting germination of seeds collected after complete ripening and those collected during different stages of fruit development belonging to selected germplasm (CPTs). In the area of investigations pre-mature collection of large size fruits is causing selective depletion of economically more valuable seeds.

3.2.3.2 Plant material

i. Terminalia chebula Retz. fruits / nuts were collected from mother trees selected as CPTs and also other trees belonging to four provenances(Table:3.1); and

ii) fruits / nuts used in the experiment belong to same fruiting season but were collected during different parts of the year commencing from the month of October and lasting up to May.

3.2.3.3 Experimental details

Treatment: I. i) Nature simulated conditions; experimental material used ripe fruits / nuts.

Earliest formed fruits i.e., from the first reproductive flush of mid May collected during next year’s first flush are about one year old. In nature ripe fruits are shed on a cushion of leaves of mother trees and gradually get exposed to elements. Simulation of what happens in nature was done and ripe fruits were sown in three replications of 30 fruits each, between April to end of August. Observations were recorded once a month. Data regarding germination that commenced during third week of July and continued up to the end of August was recorded.

Treatment: I. ii). Artificial retting condition; experimental material used ripe fruits / nuts.

Traditional practice of seed pretreatment was to bury mature fruits collected during March-May in a pit of fully retted farm yard manure for three weeks. That resulted in complete break down of pulp of fruit by microbial action and stones so released were

30
sown directly in to soil. In some nurseries of Himachal Pradesh fruits are pretreated by enclosing in containers such as two polythene bags one drawn over the other or mouth tied to encourage fungal digestion of pulp thus releasing the stone. This process is known as “bhong”. It is reportedly beneficial for ready germination of fruits that are fully ripe. Three such replications were made with 30 nuts each and sown in three different places in garden soil. Observations were timed similar to above treatment.

**Treatment: I. iii)** Artificial cracking of nuts; experimental material used ripe fruits / nuts.

Fully ripe dry fruits were de pulped by soaking in water and nuts so separated were subject to alternate soaking and drying of 48 hours each for a period of one month. Such nuts were cracked along suture line using chisel and hammer. Cracked nuts were reconstituted as they were and sown in vermiculite filled in 110 cc root-trainers. Replications set up were subject to hourly water spray using micro sprinklers under open to sky conditions. Observations were timed similar to treatment I. i).

### 3.2.3.3. a Micro-sprinkler irrigation by open mist

Irrigation for explants and seeds was provided by hit-and-splash, stirrup type of micro-sprinkler. That could generate mist (particle size 50-100 µm) when operated under pressure, say when it was put on main domestic water supply line during supply hours i.e., 5-9 AM and 5-9 PM. It could act as micro-sprinkler when pressure was low, say when connected to a 500 liters over- the- roof water tank. In the first instance the irrigation was up to 2m radius per nozzle and in the second it was up to 1.5 meters radius per nozzle. Eight micro-sprinklers could irrigate about 12 m² area with some over lapping it was used for 6 m². Many trials were done using above type of irrigation and root trainers filled with vermiculite. In the present studies wherever ‘open mist’ is mentioned it may be understood as above.

**Treatment: II. i)** Nature simulated conditions; experimental material used raw fruits / nuts.

Picking of raw fruits for murabba making regularly commences after ‘Dasehra’ festival of mid October and lasts up to the end of November every year in all the three states viz., Haryana, Punjab and Himachal Pradesh. Known weights of raw fruits were bought from farmers owning candidate plus trees (CPTs) that were identified in three provenances located in Siwalik hills of Haryana state and adjacent localities of Himachal
Pradesh where base populations were studied. To broaden the ambit of studies promising fruits from some CPTs of Himachal Pradesh were also included. Fruits were dried in shade by repeated turning to prevent fungal infection (to reduce adverse effects of early collection). In this treatment raw fruits were sown identical to treatment No. I. i) discussed above, only difference was early collection of fruits by about five months. Observations were timed similar to above treatment.

Treatment: II. ii) Condition of artificial retting; experimental material used raw fruits / nuts.

In the second treatment fruits were subject to fungal digestion of pulp and all other activities were similar to corresponding first treatment. Observations were timed similar to above treatment

Treatment: II. iii) Mechanical cracking of nuts; experimental material used raw fruits / nuts.

In this treatment after about three weeks of fruit collection and shade drying depulping was done by ‘cut and peel’ method. Pulp sticking to nuts was dislodged by soaking in water for a short while and rubbing stones against themselves. Nuts so gathered were stored with a sprinkling of contact insecticide and fungicide between October and April. During first week of April nuts were repeatedly washed in running water and alternately soaked and dried at 48 hour cycle tied in cotton cloth. Signatures of nuts were maintained throughout the operations. Emergence of radical in a few nuts signals ready conditions for germination. Nuts were mechanically cracked there after by keeping them in nut – size slots made in a log of softwood (Pinus roxburghii) using chisel and hammer along a cryptic suture line. Successfully cracked nuts with healthy seeds without any damage were reconstituted as before and sown in vermiculite filled in 110 cc HDPE composite tray of 40 cells for each CPT in three replications. Irrigation was provided as explained in paragraph on treatment No. I. i) above. Mean maximum temperature during late May and June is 36° + or - 6° Celsius and relative humidity varies between 45 - 65 %. When seedlings are six leaved or after they are shifted to 15 x 22 cm 150 gauge polyethylene bags having potting mixture of soil: silt: farm yard manure in 1:1:1 ratio.

3.2.4 Growth studies of seedlings of candidate plus trees and grafted seedlings

3.2.4.1 Introduction
Growth of seedlings of CPTs was monitored in terms of shoot growth up to third year after germination. Growth of grafted seedlings was monitored for influence of rootstock on the size of shoot, precocious flowering and fruiting and true-to-type bearing of fruits.

3.2.4.2 Experimental details

The experiments related to growth parameters of seedlings of CPTs and grafted seedlings were conducted in the nursery situated in Sector-14, Panchkula (latitude 30° 35’ 10” N / longitude 77° 05’ 05” E / 255 m amsl) during 2000 to 2003.

3.2.4.2.a Containers – polypots; polyethylene (polythene) bags & root trainers

i). polypots: One thousand seedlings (raised in Pinjore forest nursery using mixed seed-lot; one to three years old) were grown in 150 micron thick polyethylene bags. Each bag had four drain holes of 0.5 cm diameter in two tiers punched in the lower one third of height of the pots. The length x height were 15x22, 20x30 and 30x45 cm. For increasing durability large bags were made three-fold by inserting one in to other as usually they are not UV stabilized and crack under exposure to sun. The word 'polypot' used any where in the present studies may be understood as above.

ii) Root trainers: Two types of composite root trainer (RT) trays made out of high density polyethylene (HDPE) of capacity 110 ml each with 40 cells and 250 ml capacity each with 9 cells were used. Each cell has 6 ridges to train the roots and has an open but constricted end that requires to be suitably blocked to prevent escape of medium. The medium used in root trainers was horticultural grade vermiculite, sand and also standard potting mixture. In the present studies root trainer with capacity 110 or 250 ml mean above description.

3.2.4.2.b Solid rooting media – standard potting mixture, sand and vermiculite

i). Standard potting mixture consisted of mixture of equal quantities (volume / volume) farmyard manure: local soil: silt. The medium had a reaction of 6.5-7.0 pH as measured by portable pH meter by solubridge method. Seedlings were kept in open nursery.

ii). Sand of Jamuna river, rich in black mica, reputed to be non-setting type of sand when used alone.

iii). Vermiculite is a micaceous mineral, chemically, it is hydrated-magnesium-aluminum-iron-silicate when heated in a kiln to 1090° C trapped water gets expanded
forming popped up porous light medium. It occupies 90-150 kg per cubic meter; is neutral in reaction; has good buffering properties; absorbs 40-54 liters of water per cubic meter; holds nutrients for later release. For vegetative propagation 5-8 mm (No.1) was used for seed germination 0.75-1mm (No.4) was used. Wherever word ‘vermiculite’ appears it may be understood as above.

Root trainers of 110 and 250ml capacity were used as containers and medium used was standard potting mixture, sand and No.1 vermiculite.

Those seedlings that were raised from fruits / nuts collected from CPTs after transplantation in to forest areas were also kept under observation for monitoring their growth. Similarly growth of grafted seedlings, flower and fruit bearing characters were monitored at Pinjore, Sehat and Bhinwar villages 30 kilometers north of Panchkula (latitude 30° 41' 15” N / longitude 77° 10' 05” E / 1160 m amsl).

3.2.5 Studies on rooting of juvenile stem cuttings

3.2.5.1 Introduction

Pre-mature collection of fruits was draining seed-source there by adversely affecting natural regeneration. Large size fruits were receiving higher price and virtually all of them were getting exploited, leading to dysgenic pressure on base population. Natural regeneration was found to be almost negligible in the Siwalik hills of Haryana state, adjacent areas of Himachal Pradesh and Punjab. Artificial regeneration was resorted to using seed lots of any origin. To upgrade genetic quality of seedlings fruits / nuts were collected from CPTs and seedlings were grown and asexually multiplied to increase their numbers.

3.2.5.2 Plant material and PGRs

i. *Terminalia chebula,* Retz. Fruits / nuts were collected from mother trees selected as CPTs and also other trees belonging to three provenances given in Table: 3.2; and

ii) The seedlings were raised using fruits / nuts collected from CPTs and stem cuttings of up to two year old seedlings were used as explants.

3.2.5.2. a Preparation of stock solution

Stock solutions of desired concentrations of IBA, NAA, putrescine and Catechol were prepared in distilled water. Different concentrations for designed treatments were made by serial dilution technique. IBA is dissolved in a few drops of ethanol before
mixing with water, NAA is dissolved in 1 Normal solution of sodium hydroxide or directly in to distilled water that forms a milky turbid solution, putrescine a strongly smelling effervescent viscous liquid was weighed in a plastic container. It readily dissolves in water and stock solution can be stored up to 90 days in coloured bottle with firmly closed lid and catechol chips were directly soluble in water. Treatment of the cuttings with distilled water along with the same amount of ethanol / 1 Normal solution of sodium hydroxide served as control or blank. In other experiments instead of water as solvent talc powder was used. The method of dilution is explained in Table: 3.2 and the explanatory note under the table. Preparation of stock solution or stock talc powder for all experiments was done similarly.

3.2.5.3 Treatment of the cuttings

The stem cuttings were taken from nine to twenty one month – old seedlings of *Terminalia chebula* Retz. Freshly severed stem cuttings were treated with three concentrations each of IBA, NAA, Putrescine and Catechol by dipping lower 3 cm portions into the solution (long duration soaking) as follows (i) 0.1 µM (ii) 0.2 µM and (iii) 0.3 µM in water for a period of 24 hours. Cuttings were planted and exposed to natural conditions obtaining in Panchkula except for hourly mist irrigation. At fortnightly interval the planting medium was soaked to saturation with 0.03 % bavistin (carbendazim) a broad spectrum systemic fungicide.

Table: 3.2 Plant growth regulators (PGRs) and other chemicals used in experiments

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of PGR/chemical</th>
<th>Chemical Equation</th>
<th>Molecular Weight</th>
<th>Initial solvent Used</th>
<th>Diluting solvent</th>
<th>Level of Dilution In µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IBA</td>
<td>C₁₂H₁₃NO₂</td>
<td>203.24</td>
<td>Ethanol</td>
<td>Water</td>
<td>0.1;0.2;0.3</td>
</tr>
<tr>
<td>2</td>
<td>NAA</td>
<td>C₁₃H₁₀O₂</td>
<td>186.21</td>
<td>Sodium hydroxide</td>
<td>Water</td>
<td>0.1;0.2;0.3</td>
</tr>
<tr>
<td>3</td>
<td>Catechol</td>
<td>C₄H₄(OH)₂</td>
<td>110.11</td>
<td>Water</td>
<td>Water</td>
<td>0.2;0.3;0.4</td>
</tr>
<tr>
<td>4</td>
<td>Putrescine</td>
<td>C₄H₁₂N₂</td>
<td>88.15</td>
<td>Water</td>
<td>Water</td>
<td>0.1;0.2;0.3</td>
</tr>
<tr>
<td>5</td>
<td>Boric acid</td>
<td>H₃Bo₃</td>
<td>61.83</td>
<td>Water</td>
<td>Water</td>
<td>0.10;0.25</td>
</tr>
</tbody>
</table>
Explanatory note on PGRs and chemicals used to table 3.2

Indole butyric acid (IBA) 203.24 is the molecular weight; 203.24 milligram in 1000 millilitres of water is one molar solution.

Naphthalene acetic acid NAA: 186.21 g is the molecular weight; 186.21 milligram in 1000 millilitres of water is one millimolar solution.

Catechol is 1,2-dihydroxy benzene with a molecular weight of 110.11 g; when 0.1101 gram or 110 milligram is dissolved in 1000 ml of water it gives one millimolar solution.

Putrescine is 1, 4 diamino butane (or tetra methylene diamine) an effervescent viscous liquid always to be refrigerated it is soluble in water and can be kept in solution with a leak proof lid. It is desirable to make fresh solution to avoid errors owing to storage losses. Molecular weight is 88.15 grams. 0.08815 mg dissolved in 1000 ml water gives millimolar solution.

Boric acid has a molecular weight of 61.83 grams; by dissolving 61.83 grams in 1000 ml of solvent (water) we get one molar solution. For getting one millimolar solution 61.83 g are divided by 1000; we get 0.06183 milligrams. By dissolving 0.06183 milligrams in 1000 ml solvent we get 1 millimolar solution.

3.2.6 Studies on rooting of mature stem cuttings
3.2.6.1 Introduction

Seedlings begin to bear fruits commencing from third year if they have attained 8 cm diameter at the collar region. But, commercial bearing begins after 6 years when pole sized tree has formed sufficiently large crown. Seedlings raised from fruits gathered from CPTs are not always true-to-type. Field evidence was collected to study the behaviour of F₁, regarding qualities of fruit, from four locations viz., Mandhana, (Panchkula district) Kothi, (Bilaspur district) Jamun ki sire (Nahan district) and Philorhi (Nahan district) in Himachal Pradesh. Trees that were raised using seeds collected from large fruit bearing trees behaved as under. At Mandhna they were true-to-type; at Kothi fruit size borne by F₁ was smaller by 40% in all the trees when compared to the parent while medicinal properties (quality) for which the tree is reputed, continued to be maintained. At Jamun ki sire fruit size decreased by 35% in F₁ progeny. At Philorhi the F₁ progeny showed mixed results out of ten F₁ trees, 2 trees were true-to-type as far as fruit traits are concerned. They
produced identical fruits both in size and quality, but the rest of the eight trees produced 
false-to-type fruits both in size and quality.

Above circumstantial field evidence indicated that the wild population is usually 
heterozygous and the filial generation shows difference in fruit size. To get uniformly 
large fruits in precocious manner root induction in stem cuttings was tried using various 
PGRs to raise genetically superior seedlings.

3.2.6.2 Plant material

One, two and three year old branches of up to 1 cm, 2-3 cm 3-5 cm diameter 
respectively were severed from the selected mature trees and cuttings measuring 15, 20 
and 30 cm respectively were prepared.

3.2.6.2. a Preparation of stock solution

Solutions of listed PGRs and other chemicals used were prepared as explained in 
paragraph 3.2.5.2.a read with table: 3. 2 and explanatory note given under the table

3.2.6.3. Experimental details-treatments of cuttings.

Freshly cut stem cuttings were brought wrapped in moist gunny bags from the tree 
to the location where experiment was to be set to prevent loss of endogenous moisture.

3.2.6.3. a Surface sterilization and wax capping

Surface sterilization and wax capping was done in four stages as explained under. 
i). Neutral liquid detergent viz., Teepol / labolene 3% volume / volume was used to give 
dust free surface to the cuttings by shaking the cuttings under water containing teepol / 
labolene. Above cuttings were washed thrice in running water.
ii). In water solution of 0.01 percent mercuric chloride or emesan (methyle mercuric 
chloride) for a period of 05 minutes; followed by a thorough wash in fresh water.
iii). Treatment by dipping in 0.03 percent water solution of bavistin (carbendazim), a 
broad spectrum systemic fungicide for a period of 10 minutes.
iv). Subsequent to that upper 2-3 cm of explants were dipped in molten wax used as a 
sealant.

3.2.6.3. b PGR treatment to explants

Cuttings thus prepared were treated to designed concentrations of PGRs by dipping 
lower 3-5 cm portion in to the solution for a period of 24 hours. These treated cuttings 
were planted either in sand beds / composite HDPE root trainers containing horticultural 
grade 4-8 mm vermiculite explained in paragraph 3.2.4.2.b.
The set up was subjected to hourly irrigation for five minutes duration by micro-sprinklers under an open mist situation as explained in paragraph 3.2.3.3.a above. Those cuttings with developed shoots and roots were transplanted in to beds or polythene bags containing garden soil after suitable hardening. Identity of the treatment was maintained using aluminum tags written over with stylus.

3.2.7 Studies on root induction of rejuvenated stem cuttings

3.2.7.1 Introduction

Mature stem cuttings so far have responded to the extent of callus formation by PGR stimulation. Induction of juvenility by heading back a mature tree in some species that are difficult or obstinate to root using mature explants, have given operational success eg., *Eucalyptus tereticornis* (Rao, 2001) *Pinus radiata* (Zobel, 1988).

3.2.7.2 Plant Material

One mature tree aged about 60 years was felled in the village Mandhana to induce coppiced shoots. Felling was done before summer to induce stool / coppice shoots, but the stump did not sprout. Consequent to this in another tree a branch that was growing at the collar portion of the tree was cut and vigorous branches grew just below the cut end. They were allowed to grow for 45 days and the first crop of shoot cuttings were collected during the month of mid June and were used as explants.

Leaf surface was reduced by two-third of the length by cutting tip backward to reduce overlapping of leaves when they are planted in root trainers. At least two leaves were retained on each cutting to give explant large enough photosynthetic area. Lower portion of the branch that is woody and terminal portion that has only soft tissues was not used and only middle portion was used for preparation of the cuttings.

3.2.7.3 Preparation of the stock talc powder

Stock talc powder of IBA and putrescine were prepared; IBA stock was prepared by mixing known weight of very fine crystals (powder) in 10 grams of talc powder and was then made into known weight with talc. Known weight of putrescine (effervescent viscous liquid) is mixed with talc and final weight was made up by addition of required quantity of talc. All stock mixtures were stored in refrigerator using opaque non reactive plastic containers with air tight lids. The mixtures of desired concentrations required for treatment were prepared by suitably diluting the stock mixture with talc. Treatment of the cuttings with talc without any PGRs served as the control. For keeping leafy explants
within the mist chamber minimum time is lost between severance from the donor and planting, considering this a quick dip treatment with talc was more convenient treatment compared to 24 hour soaking that was used in most of other trials.

3.2.7.4 Method of treatments of the cuttings.

Freshly cut stem cuttings were brought in a bucket containing water with their cut ends dipped in 0.03 % of bavistin. Surface sterilization was done in two stages as explained in paragraph number 3.2.6.3a above. The set up was kept in a mist chamber in which relative humidity was maintained at 95 % and temperature 42 ° Celsius with cycles of spray of water by micro - sprinklers as under. Drainage through the rooting medium is fairly rapid and no water logging occurs. Most of the difficult to root plant species root under stressed conditions of mist chamber as sterility, high temperature and humidity are increased to stress inducing levels that forces plant to higher metabolic rates of virtual rain forest conditions. Explants are kept in this condition for a period of 45 days and they are taken out to hardening area for about 15 days and beyond that they are kept in open to sky nursery beds. Seedlings are given foliar spray of major and minor nutrients but after contact with soil nutrient supplement is dis-continued so as to make seedling self-reliant. Misting regime to maintain 95 % relative humidity and 40 ± 2 ° Celsius temperature when external maximum shade temperature is 35 ° Celsius and minimum relative humidity is between 20-35 %. Please see misting regime in Table: 3.3

3.2.8 Studies on root induction of lopping-induced epicormic branches

3.2.8.1 Introduction

Various phenophases of *Terminalia chebula* Retz. were reasonably well determined in Siwalik hills. Sprouting in mature trees occurs commencing from last week of April followed by reproductive growth. But in trees that are lopped severely during October i.e., at the time of raw-fruit collection, epicormic sprouting occurs during autumn (November) and persists in a stagnant form during winter (December-February) and begins to grow during summer (May) these lopping induced sprouts were used as explants during middle of February for adventitious root initiation.

3.2.8.2 Plant material

Lopping induced stem cuttings were collected from trees that grow in Mandhana village during middle of February. Leafy explants were transported during forenoon in bavistin containing water bath kept in a covered vehicle. Most of the sprouts are golden
yellow in colour at this stage and sprout from dormant buds in clusters. They are quite vigorous in growth but lignification was not much in evidence and they tend to be soft except at the lowermost portion.

3.2.8.3 Experimental details

3.2.8.3. a Preparation of the stock talc powder

Stock talc powder of IBA, NAA, and Boric Acid were prepared similar to details given in paragraph number 3.2.7.3 read with table number 3.2 and explanatory note.

3.2.8.3. b Method of treatment of the cuttings.

Surface sterilization was done as described in paragraph number 3.2.6.3.a above. Cuttings thus prepared were treated to designed concentrations of PGRs by dipping lower 2-3 cm portion in to the talc mixture containing the PGR. These treated cuttings were planted in composite HDPE root trainers containing horticultural grade 4-8 mm size vermiculite.
Table 3.3 Mist regime that is required to maintain 95% relative humidity and 40 ± 2°C temperature when external temperature is 35°C and RH is 20-35%.

<table>
<thead>
<tr>
<th>From</th>
<th>To</th>
<th>Spray; seconds</th>
<th>Non-spray: Minutes</th>
<th>Duration Hours Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>07.30</td>
<td>09.00</td>
<td>15</td>
<td>30</td>
<td>1. 30</td>
</tr>
<tr>
<td>09.00</td>
<td>11.00</td>
<td>15</td>
<td>10</td>
<td>2. 00</td>
</tr>
<tr>
<td>11.00</td>
<td>15.00</td>
<td>12</td>
<td>05</td>
<td>4. 00</td>
</tr>
<tr>
<td>15.00</td>
<td>17.00</td>
<td>15</td>
<td>07</td>
<td>2. 00</td>
</tr>
<tr>
<td>17.00</td>
<td>19.00</td>
<td>15</td>
<td>30</td>
<td>2. 00</td>
</tr>
<tr>
<td>19.00</td>
<td>07.30</td>
<td>00</td>
<td>-</td>
<td>12. 30</td>
</tr>
<tr>
<td>24 hours</td>
<td></td>
<td></td>
<td></td>
<td>24. 00</td>
</tr>
</tbody>
</table>
3.2.9 Studies on air-layering of juvenile *Terminalia chebula* Retz.

3.2.9.1 Introduction

One of the horticultural methods of vegetative propagation to produce true-to-type individuals is air-layering. In this method, terminal portions of stem of a selected individual, usually a CPT are induced to produce roots by excision of bark and application of PGRs while it is still attached to the parent tree by wood (xylem). Number of air-layers in the first cycle is limited to capacity of crown of the mother plant, but once a successful air-layer treatment is established *ex situ* collection can be made and seedlings can be produced in geometric progression.

3.2.9.2 Plant material

Plant material that was used; half-sib seedlings from nuts of CPTs and also seedlings raised from any seed-lot collected from forest nursery. Age of the seedlings 2-5 years, irrespective of seed source used. Actual part used was 0.5-3 cm diameter branches both main and side branches were used. Age at the point of air-layer was less than four years and more often between 1-3 years.

3.2.9.3 Experimental details

3.2.9.3. a Preparation of the stock talc powder

Stock talc powder of IBA, NAA, and Boric Acid were prepared as discussed in paragraph number 3.2.7.3 above.

3.2.9.3. b Method of air layering

Portions of stem that were developing lenticels immediately below the vigorously growing points were chosen for air layering. Plants had more than a dozen leaves ahead of point of air-layer. That point of transition has a favourable combination of both vigour and mechanical strength. Bark up to 1.5 -2.0 times the diameter of the branch was peeled by girdling at two places using sterilized budding knife. Phloem elements sticking to the wood were scraped clean to prevent phloem ‘bridge’ formation between two girdled ends. At the girdled distal end PGR application was made using talc ‘carrier’ powder using sterile cotton gauze that was used as a multilayered bandage. Also the peeled portion was smeared with ‘carrier talc’ to slow down ‘browning’ caused by oxidation. Transparent polyethylene sheet of 150 microns was used as a sleeve to hold rooting medium viz., vermiculite. Sufficient quantity of vermiculite up to 125 cc per air-layer that forms a complete surround at the girdle and also 2.5 cm either way beyond the girdle was tied.
using 10 No. cotton thread (thick) in an air tight fashion. Air tightness gets revealed within a matter of 60 minutes by accumulation of water droplets on the inside of the polyethylene sleeve during active growing season. Observations were made once in ten days beginning from the date of air-layering.

3.2.9.2 Air layering of mature *Terminalia chebula* Retz.

3.2.9.2.1 Introduction

*Ex situ* conservation of economically more valuable germplasm and also mass multiplication holds the key to making alternative indigenous tree species available to tree farmers. As the ‘elite’ trees are very few and far between with an intensity of 2 trees per thousand trees and their fruits are preferably bought by connoisseurs increasing their numbers by producing air layered plants was attempted. Initially trials were laid out in all the four quarters of the year but later on the basis of experience gained it was restricted to spring (February-March), late summer (July-September i.e., monsoon) and early fall(October).

3.2.9.2.2 Plant material

Plant material that was used; mature trees both CPTs and wild variety occurring naturally in Morni hills, Philorhi and also those planted in sector 31-D of Chandigarh. Age of the trees varied between 40-80 years. Actual part used was 0.5-4 cm diameter branches both main and side branches were used. Age at the point of air-layer was less than five years and more often between 1-3 years. The tree is lopped repeatedly and produces clusters of epicormic branches being vigorous and straight growing they were used for air-layering.

3.2.9.2.3 Preparation of the stock talc powder

Stock talc powder of IBA, NAA, and Boric Acid were prepared as discussed in paragraph number 3.2.7.3.above. As most of the trials made earlier had indicated that root initiation process was not fully accomplished in mature plants by using permutations and combinations as shown in treatments of juvenile air-layers some more treatments were also included for air-layering mature trees using various concentrations of IBA as indicated in the table.

3.2.9.2.4 Method of air layering

Method of air layering was similar to that used in paragraph number 3.2.9.3.b above.
3.2.10 Studies on grafting of soft and hardwood

3.2.10.1 Introduction

Grafting has been a very practical and low cost conservation technique for \textit{ex situ} collection of germplasm. It has been used from times immemorial in horticultural practices. Many of the fruit plants, with superior fruit qualities owe their origin to grafting practices. Also, grafting is a ready method of proving true–to–type traits of a candidate germplasm, that would otherwise take longer time, particularly for study of fruit characters. Advantage of almost fully established root stock can be availed by ‘top-working’ there by reducing waiting period in establishment of fruit / seed orchards by grafting. One precondition of grafted seed orchards is very high intensity of management particularly during its initial stages. If this is denied the perpetual tendency of root stock to overpower the scion gets expressed. Greater chances of this happening is there in forestry practices as the tract is wild and the grafted plant is likely to get exposed to vagaries of weather. Periods of reduced supply of factors essential for growth may trigger reversion. This method has been resorted to in present studies as a last resort for \textit{ex situ} conservation of germplasm as other macropropagation methods had not responded for mass multiplication of mature explants.

3.2.10.2 Plant Material

3.2.10.2. a Stock plant

Description of Seedlings used as stock plants. Nursery raised seedlings as described in Table: 3 were used as stock plants.

\textbf{Table: 3.4 Details of stock plant}

<table>
<thead>
<tr>
<th>Age in Months</th>
<th>Size of polypot (cm)</th>
<th>Collar Diameter (cm)</th>
<th>Top Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>12 x 22</td>
<td>0.4</td>
<td>25</td>
</tr>
<tr>
<td>12</td>
<td>20 x 30</td>
<td>0.8</td>
<td>50</td>
</tr>
<tr>
<td>24</td>
<td>30 x 45</td>
<td>1.5</td>
<td>75</td>
</tr>
</tbody>
</table>

3.2.10.2. b Scion

The budwood was collected from mature CPTs of age varying from 25 - 100 © years. The collection was done commencing from the month of June and continued up to
end of July. It belonged to four different age classes as explained under viz.,
(i) current year’s branch, sprouted during last week of April and on an average one- and-
half- months old, that was uniformly green without lenticels visible to naked eye
(softwood);
(ii) corresponding to above previous season’s vegetative branch aged thirteen - and - half-
months old that was greenish brown with lenticels visible to naked eye (softwood);
(iii) branches that sprout after fruiting that are aged eight - and -half - months old
(softwood); and
(iv) vigorous and straight growing branches as a result of lopping that are up to 3 years old
that measured up to 2.5 cm diameter; also epicormic water shoots (hardwood).

3.2.10.3 Method of grafting

Pretreatment of budwood by surface sterilization as explained in paragraph number
3.2.6.3.a was done. Residue of fungicide was washed in running water and explants
conserved with freshened cut ends immersed in containers having distilled water.

Scions of appropriate size suited to diameter of stock plant at the point of proposed
graft union were made. Sharp knife/ razor blade was used to shape the scion in the form of
a peg symmetrical in one plane. The knife / razor blade was surface treated with ethanol
before every operation to prevent possible contamination. A matching cut was made in the
stock plant and peg of scion was snuggly fitted in to the matching cut. The stock and
scion at the point of union was meticulously matched to ensure juxtaposition of cambium.
The point of union was tightly wrapped around with 2 cm wide strips of low density
polyethylene (LDPE). Multiple rounds of wrapping were done to exclude air spaces where
stock and scion had cut surfaces. Loose end was secured by a half knot.

The grafted plant was enclosed in a LDPE bag that was used as a cap. The size of
the cap was 15 x 20 cm, in the beginning it was longitudinally folded thrice, central fold
held the grafted plant as the bud break and elongation occurred rest of two third of bag
was unfurled. Lower portion of the bag was loosely constricted either with staples or with
elastic rubber bands. Care was taken to allow free access to circulation of air. The cap was
removed after the branch started growing vigorously. The capped grafted plant was left
open to sky during 5 p.m to 10 a.m (cooler part of the day) and covered with 75 % shade
net during 10 a.m to 5 p.m (hotter part of the day). Sprouting was recorded at ten days
interval and the strips of tape used at graft union / joint was cut and removed after a month
or later to ensure proper union.

To reduce water loss some water can also be artificially introduced in to above arrangement that has been considered as a separate treatment. Once the bud break occurs and elongation begins water bubble causes leaf fall, it is better to shift to cap without water as discussed above soon after elongation of shoot begins. Pre - sprouted stem cuttings that are obstinate to root can be grafted to stock plants by this method.

3.2.10.4 Grafting of sprouts induced on stem cuttings

The mature explants collected from elite trees were being treated to various PGRs for root induction studies were used in this study as they profusely sprouted and gave shoots but failed to root beyond minor callus formation. The sprouts at the time of grafting were thirty to forty five days old and mostly contained soft tissue, they were carefully severed with sterilized razor blade from the stem and shaped in the form of a wedge and grafted on to top portion of one year old seedling as top mounts. Three treatments of scions viz., apical portion only, middle sprout and entire sprout were used in the experiment. In this experiment the leaves were retained as they were very small and delicate and removal would have harmed the scion. Grafts were capped with LDPE bag as above and similarly treated. Graft union was monitored on daily basis and the binding tape was removed as explained above.

3.3 Arjun (Terminalia arjuna Wright & Arn.)

3.3.1 Introduction

Present investigations were undertaken with the plant species Terminalia arjuna W&A. It is a peninsular Indian species that occurs along river courses as first row of trees. It has been introduced in north India as an avenue tree and grows as an escape in some moist locations, such as stream sides. It has been described in the Forest Flora for the Punjab with Hazara and Delhi and Flora of Haryana (Parker, 1950; Kumar, 2000). It is a long-lived, hardy, summer deciduous tree that can grow in secondary salinized locations in plains. Bark is reputed to possess anti cholesterol properties and is used in Ayurveda. Leaves are valuable fodder for cattle and rearing tassar-silk larvae. It is also used as industrial raw material for manufacture of oxalic acid. It is a multi-use tree as wood can be used as timber and firewood.

3.3.1. a The following major areas were studied in case of ‘arjun’:

46
• Survey of base population in five provenances was carried out. Identification of healthy phenotypic superior trees that have shown higher rate of growth, called as candidate plus trees (CPTs) was done.
• Tree-wise (CPT-wise) collection of ripe fruits that were used for germination studies.
• Raised tree-wise (CPT-wise) seedlings for assessing genetic variation and selection of promising ones for carrying out seed production areas.
• Carried out clonal propagation (vegetative), of juvenile and mature stem cuttings, especially those obtained from CPTs.

3.3.2 Survey of base population
3.3.2.1 Introduction
Natural populations of arjun were not found in Siwalik and other areas of Haryana. Therefore, introduced exotic plantations were studied as they occur in provenances i) Chandigarh- Panchkula, ii) Kurukshetra, iii) Karnal, iv) Kala Aamb and v) New Delhi.

3.3.2.2 Plant Material
Avenue plantations as indicated in Table: 3.5 were measured over bark in cm at 1.37 meters height above ground level. Those trees that were more than 100% of the adjacent trees-termed as ‘satellites’ and had a clear trunk and crown were selected as CPTs. Growth vigour as expressed in size of the trunk of same-aged plantations was the criterion of the selection of candidate plus trees (CPTs). At the time of selection, to reduce the play of site factors (environment) those phenotypic superior trees that had discernible advantages. Out of 960 trees studied 23 trees were short - listed for collection of fruits for further studies of germination and natural variation in qualities of seedlings. Thus the selection intensity works out to 24 trees for 1000 trees studied.
### Table: 3.5 Selection of CPTs of *Terminalia arjuna* W& A.

<table>
<thead>
<tr>
<th>Name of provenance</th>
<th>District</th>
<th>Geographic location</th>
<th>Base Population (No.)</th>
<th>CPTs Selected (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Chandigarh - Panchkula</td>
<td>Chandigarh (UT) Panchkula</td>
<td>29° 30'' 76° 30''</td>
<td>550</td>
<td>9</td>
</tr>
<tr>
<td>ii) Kurukshetra</td>
<td>Kurukshetra</td>
<td>29° 55'' 76° 15''</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>iii) Karnal</td>
<td>Karnal</td>
<td>29° 15'' 76° 30''</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>iv) Kala Aamb</td>
<td>Ambala</td>
<td>27° 15'' 76° 30''</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td>v) New Delhi</td>
<td>Delhi Metropolis</td>
<td>29° 00'' 77° 15''</td>
<td>200</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>960</strong></td>
<td><strong>23</strong></td>
</tr>
</tbody>
</table>
3.3.3 Germination studies of seeds of *Terminalia arjuna* W&A.

3.3.3.1 Introduction

*Terminalia arjuna* trees flower in north Indian plains commencing from second half of May and flowering lasts through the month of June every year. Fruit formation is profuse but progressively decreases in older trees. Fruit is a five winged drupe, has a horny endocarp and corky and porous mesocarp. This species is naturally distributed in peninsular India its favourite habitat is along stream courses. There fruit /seed ripening precedes monsoon and is shed along with pre-monsoon showers or slightly earlier. With increase in the level of the flood waters fruits get afloat as their density is less than that of water and get transported over long distances (dispersal). They get deposited along the high/highest flood level (HFL) along river terraces with rich vegetable debris. The decaying vegetable debris provides seed-bed conditions for fruits to germinate. Germination begins almost within ten days and to cover up contingency of loss of germinated seed by fresh flood waters cohorts of fruits are produced by the tree.

3.3.3.2 Plant material

Ripe fruits collected from CPTs were used as experimental material.

3.3.3.3 Experimental details

Treatment: i) Nature simulated conditions; experimental material used ripe fruits that are naturally shed by the CPT.

Flowering in *Terminalia arjuna* Wright and Arn. occurs during late spring-early summer part of the year and fruits mature during next summer just before monsoon i.e., after 12 months. The intensity of flowering and fruiting is very high and fruits are borne almost every year. In nature ripe fruits are shed on the ground below the tree along fresh water course. Simulation of what happens in nature was done and ripe fruits were retained in 1 m² quadrats in three replications of 30 seeds each during the month of June. Observations were recorded thrice a month. Data regarding germination that commences during July and continues up to the end of August was recorded.

Treatment: ii) Pretreatment in shallow pits with water.

Fruits of CPTs were collected tree – wise and placed in 20-25 cm deep shallow pits. They were turned once a while to completely soak them in water and those of the germinating fruits out of thirty fruits per replication were sown into root trainers.
Pretreated seeds were sown in a time series commencing from July first week up to the end of August in 110 ml root trainers filled with sterile vermiculite. Above set up was subject to intermittent spray of water through misting nozzle of 5 minutes duration for every hour during day time under open to sky situation. The first spray every day was for not less than 30 minutes to completely soak vermiculite / soil to saturation. Mean maximum temperature during July is 36 ° + or - 6 ° Celsius and relative humidity varies between 65 - 85 %. Observations on germination and growth were recorded once in ten days in three replications with sample size of 30.

Treatment: iii) Ripe fruits were heaped under shade of the tree and kept moist by frequent irrigation by rose can

Fully ripe fruits that were shed naturally by CPTs were heaped under the shade of a tree and kept wet by irrigating with rose can at two hourly intervals. Commencing from the month of June this treatment was continued during July after the rains set in watering was discontinued. At regular interval of 10 days, fruits that germinated were counted and sown in to root trainers. They were exposed to micro-sprinkler irrigation. Observations were timed similar to treatment i above.

Treatment: iv) Sowing of fruits in to poly-pots filled with planting medium (sand : soil: vermi-compost 1:1:0.5 v/v); treated as control.

Standard nursery potting mixture consisting of sand, good quality soil and vermi-compost were filled in to 20x30 cm polyethylene bags of 150 micron thickness. They are arranged in a sunken beds measuring 1m x 10 m. Fruits are pre-treated by soaking in water for 48 hours by steeping gunny bags filled with fruits in masonry tank. Said set up is irrigated by rose can 10 times a day. Observations are recorded similar to above treatments.

3.3.4 Studies on effect of PGRs on rooting and sprouting of juvenile stem cuttings of *Terminalia arjuna*, Wright and Arn.

3.3.4.1 Introduction

*Terminalia arjuna* Wright and Arn. Is a peninsular Indian species that has been introduced and almost naturalized in north India. Very old trees are present in Siwalik hills of Jammu and Kangra valley (Parker, 1950). Natural regeneration of this species in north India has been found to be retarded as the fresh water streams along which it grows are youthful and fail to deposit as much silt and organic matter in hills as mature streams of
peninsular India. The seedbed conditions required for germination and establishment of seedlings are absent wherever it is present regeneration is adequate.

Salinity and water logging are a part of natural habitat where the tree grows; thus the tree has evolved a natural adaptation for growth along wet salt rich sites. In semi-arid warm tropical climate intensive irrigation with non-judicious water use has caused secondary salinized sites, this species is naturally adapted to such conditions. As there is natural variation in the vigour of the plant, fast growing trees can be selected and the best grade of seedlings can be multiplied for raising plantations. In this direction vegetative propagation holds promise hence the studies.

### 3.3.4.2 Material and method

This investigation was carried out on juvenile stem cuttings of *Terminalia arjuna* W&A. obtained from six months to one year old nursery raised seedlings. Tallest (top height in cm) and sturdiest (collar diameter in cm) were selected during four quarters of the year beginning with October, January, April, and July. Leaves when present were defoliated and sub - apical main stem (usually single stemmed at this stage of growth) was cut in to 10 cm long pieces. The apical portion that is essentially parenchymatous was discarded. Explants were surface sterilized and top portion was sealed as explained in paragraph 3.2.6.3.a.

Cuttings were treated to low concentration long duration end-soaking of 24 hours. Four different treatments of indolebutyric acid (IBA) 2 μ Mols; IBA and naphthalene acetic acid (NAA) 2 μ Mols each; IBA and pyro catechol 2 μ Mols each; and pyro-catechol; and putresene (polyamine) 2 μ Mols; and a control was used in the experiment. The treated explants were set in rooting medium subject to intermittent spray of water under ‘open misting’.

The decapitated juvenile donor plants vigorously sprout and were not lost in the process. Explants sprout along with other seedlings during last week of April and first week of May when out-door shade temperature is 36° or more.

### 3.3.5 Studies on root induction in mature stem cuttings of *Terminalia arjuna* W&A.

#### 3.3.5.1 Introduction

*Terminalia arjuna* W & A. being raised in the form of amenity plantations and farm / agroforestry situations in north India especially in Haryana, Punjab and Delhi. There is natural variation in the vigour of the plant hence in the size of same-aged trees
and CPTs have been marked for collection of seeds / fruits and also explants for root induction studies. It is a tree that is pollinated by insects dilution of ‘elite’ traits is a distinct possibility when the tree out breeds. Ex situ conservation of CPTs can be done if explants are asexually propagated, therefore, the experiment.

3.3.5.2 Material and method

3.3.5.2 a Plant Material

This investigation was undertaken with the plant species *Terminalia arjuna*, *Wright and Arn* which belongs to difficult –to- root category. Stem cuttings from mature CPTs were collected during the month of May, those cuttings that were about 3 cm in diameter at the base; were one year old and vigorous in growth were preferred. Collection was so timed that the bud break was about to occur. The explants were surface sterilized and top portion sealed by dipping in to molten wax as explained in paragraph 3.2.6.3.a before. Every replication was set in either HDPE composite tray of forty cells; each of 110 ml capacity filled with vermiculite medium or sterilized Yamuna river sand.

3.3.5.2. b Preparation of the stock solution

Stock solutions of IBA, NAA, Putrescine, Catechol and Boric Acid were prepared; IBA as discussed in paragraph 3.2.5.2.a read with Table: 3.2 and note there under.

3.3.5.2. c Treatments to cuttings

Freshly cut stem cuttings were brought wrapped in moist gunny bags from the tree to location where experiment was to be set to prevent loss of endogenous moisture. Surface sterilization and wax capping was done in four stages as explained in paragraph 3.2.6.3.a above. Cuttings thus prepared were treated to designed concentrations of PGRs by dipping lower 3-5 cm portion in to the solution for a period of 24 hours. These treated cuttings were planted either in sand beds (gray biotite mica rich Yamuna river sand) / composite HDPE root trainers containing horticultural grade 4-8 mm vermiculite. The set up was subjected to hourly irrigation by 'open mist’as explained in paragraph 3.2.3.3.a above. Those cuttings with developed shoots and roots were transplanted in to beds or polythene bags containing garden soil after suitable hardening. Identity of the treatment was maintained using aluminum tags written over with stylus.
3.4 The Indian elm tree (*Holoptelea integrifolia* Planch.) Kanji wood, pahari papri

3.4.1 Introduction

*Holoptelea integrifolia* Planch. Greek *holos*, entire, *ptelea*, the elm; the tree differs from *Ulmus wallichiana* in having entire leaves (Parker, 1956). It is a large, long-lived, handsome shady tree that requires very little attention after it is few years old. It is distributed in hills of Aravalli and Siwalik ranges. It can grow into a very large size tree in sand dunes and also along storm water drains of Siwalik ranges. It is winter deciduous and therefore has compatibility with agroforestry systems of wheat-paddy rotation as practiced in north Indian plains. The leaves are not good fodder as they emit strong, unpleasant smell that explains why it is not selectively browsed. But in fodder deficient areas cattle are forced to eat cut and carry fodder under duress. Once initial hesitation is over come, thereafter cattle do not discriminate against this fodder. Wood is moderately hard, diffuse porous and resembles that of *Terminalia myriocarpa* the ‘holak’ that is being extensively used in and around Chandigarh as timber in building construction.

In Haryana, Punjab, Uttarakhal and Uttar Pradesh agroforestry is practiced extensively. Only two exotic tree species viz., *Eucalyptus tereticornis* Smith and *Populus deltoides* Gaertem. are being used. Diversification of species, particularly indigenous species has become very important. One of the major requirements for getting considered as a likely candidate under agroforestry systems is winter leafless condition. Absence of shade during winter wheat crop makes this species compatible with agroforestry. Being a hardy tree and of universal application in sandy areas, plains, rocky hills of Aravallis and Siwaliks it can be held out as a possible indigenous candidate to replace above named exotic species. It can be raised in large numbers very easily if fresh seeds are sown immediately after collection.

3.4.1. a The following major areas were studied in case of pahari papri or Kanji

- Survey of base population in four provenances was done. Identification of healthy phenotypic superior trees that have shown higher rate of growth and good trunk characters called as candidate plus trees (CPTs) was carried out.
- Collected tree-wise (CPT-wise) ripe fruits that were used for germination studies.
Carried out clonal propagation (vegetative), of juvenile and mature stem cuttings, especially those obtained from CPTs.

3.4.2 Survey of base population

CPTs were selected from Kalesar, (Yamuna Nagar) Samana, (Karnal) Bhondsi (Gurgaon) and Naolta (Panchkula) on phenotypic basis. Preference was given to same aged trees on the basis of vigour as expressed in girth and height of mature tree. This basis was not evident in younger plantations of this species up to 7 years, beyond that a few stems were found to be slightly larger in size compared to other trees. Considering this fact trees beyond 40 years of age and natural trees of exceptional bole and stem characters have been chosen and are tabulated in Table : 3.6.

3.4.3 Germination studies of *Holoptelea integrifolia*

3.4.3.1 Introduction

*Holoptelea integrifolia* Planch. trees flower once in a year during late spring / summer, flowering is followed by vegetative growth. The light fruits are shed in the midst of hottest part of the year thereafter the tree clothes in new leaves. Seeds are viable for a short period of up to 60 days and begin to lose viability. Gynoecium is reduced in to a stalked single ovuled-samaroid fruit with a fringe-wing that initially has a forked style and plumose stigma. Initial slow growth is characteristic of this species and continues up to about 7 years thereafter it comes to form and grows rapidly with a strong root system. Objective of the experiment was to study factors affecting germination with a view to solve the problem of meagre natural regeneration.
Table: 3.6 Geographic locations of CPTs selected

<table>
<thead>
<tr>
<th>Name of provenance</th>
<th>Geographic location</th>
<th>Size of base</th>
<th>No. of candidate plus trees selected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N. Latitude E. Longitude</td>
<td>Population</td>
<td></td>
</tr>
<tr>
<td>i) Kalesar</td>
<td>30° 25&quot; 77° 15&quot;</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>ii) Samana</td>
<td>30° 41&quot; 77° 10&quot;</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>iii) Bhondsi</td>
<td>30° 35&quot; 77° 05&quot;</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>iv) Naolta</td>
<td>20</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>11 (8%)</td>
<td></td>
</tr>
</tbody>
</table>
The light fruits are shed in the midst of hottest part of the year thereafter the tree clothes in new leaves. Seeds are viable for a short period of up to 60 days and begin to lose viability. Gynoecium is reduced into a stalked single ovuled samaroid fruit with a fringe-wing that initially has a forked style and plumose stigma. Initial slow growth is characteristic of this species and continues up to about 7 years thereafter it comes to form and grows rapidly with a strong root system. Objective of the experiment was to study factors affecting germination with a view to solve the problem of meagre natural regeneration.

3.4.3.2 Material

Ripe fruits of CPTs and other trees were used in the experiment. Care was taken to collect the fruits from the branches of trees before shedding so that accidental mixing is avoided.

3.4.3.3 Experimental details

Treatment: i) Nature simulated conditions; experimental material used ripe fruits that are naturally shed by the CPT. Treated as control.

Flowering in *Holoptelea integrifolia* occurs once during late spring-early summer part of the year and fruits mature during same season well before monsoon. The intensity of flowering and fruiting is very high and fruits are borne almost every year. In nature ripe fruits are shed on the ground below the tree and along major wind direction i.e., south west. Simulation of what happens in nature was done and ripe seeds were retained in 1 m² quadrats in three replications of 30 seeds each during the month of June/ July/ August. Observations were recorded twice a month. Data regarding germination that commences during July and continues up to the end of August was recorded.

Treatment: ii) Pretreatment by alternate wetting and drying cycles.

Fruits of CPTs were collected tree – wise and placed in gunny bags and soaked overnight. After first nights soaking they were exposed to sun after draining water. This alternate soaking and drying in cycles of 12 hours was continued till the seeds began to germinate. Those of the germinating fruits out of thirty fruits per replication were sown into root trainers. Pretreated seeds were sown in a time series commencing from July first week up to the end of August in 110 ml root trainers filled with sterile vermiculite. Above set up was subject to intermittent spray of water through misting nozzle. Observations on
germination and growth were recorded once in ten days in three replications with sample size of 30.

Treatment: iii) Ripe seeds were extracted by breaking the fruit and excised seeds were sown initially on to filter paper and germinating seeds were transferred to root trainers.

Fully ripe fruits that were CPTs were collected. Seeds are encased in a flat fruit wall were excised mechanically and surface sterilized by 0.01 % mercuric chloride for 2 minutes and 2% bavistin in water for ten minutes. Thereafter they were sown in petri dishes with wet filter paper. Germinating seeds were transferred to root trainers and treated similar to above treatment.

Treatment: iv) Sowing of fruits in to poly-pots filled with planting medium (sand:soil: vermi-compost 1:1:0.5 v/v).

Standard nursery potting mixture consisting of sand, good quality soil and vermi-compost are filled in to 15x22 cm polyethylene bags of 150 gauge. They are arranged in a sunken bed measuring 1m x 10 m. Fruits are pre-treated by soaking in water for 48 hours by steeping gunny bags filled with fruits in masonry tank. Said set up is irrigated by rose can 5 times a day. Observations are recorded similar to above treatments. Within ten days germination commences by extension of radical first, the shoot portion organizes simultaneously.

3.4.4 Effect of PGRs on rooting and sprouting of juvenile stem cuttings of *Holoptelea integrifolia* Planch.

3.4.4.1 Introduction

*Holoptelea integrifolia* Planch. is a tropical ubiquitous species found in Siwaliks and Aravallis. Being one of the hardy species in areas subjected to biotic pressure it tends fight back and holds its fort till all other species have vanished from the area. This is the last struggling and surviving species in biodegraded areas of Aravallis and Siwaliks. The seedbed conditions required for germination and establishment of seedlings are generally not present wherever they exist regeneration is adequate.

But studies have indicated that there is not much of natural variation ,even if it exists, that gets expressed by about 8th year after plantation. A through verification of 5000 seedlings in three forest nurseries of the Panchkula district viz., Pinjore, Mouli and Shapur
was done and most vigorous one year old seedlings were selected. Cuttings from those plants served as explants for this work. Objectives of the experiment are as under:

i) To determine the effect of PGRs on rooting of juvenile stem cuttings to increase availability of good quality seedlings; and

ii) verify suitability of vegetatively propagated seedlings as possible alternative to seed-origin seedlings.

3.4.4.2 Material

This investigation was carried out on juvenile stem cuttings of *Holoptelea integrifolia* Planch. obtained from twelve month old nursery raised seedlings. Tallest (top height in cm) and sturdiest (collar diameter in cm) were selected and planted during May. The main stem that was used as plant were cut in to 10 cm long pieces, small side shoots were rejected. The soft apical portion that is essentially parenchymatous was discarded. Explants were surface sterilized and top portion was sealed as explained elsewhere in this thesis.

3.4.4.3 Experimental details

The explants were treated as explained under paragraph 3.2.5.3 above.

3.4.5 Studies on root induction in mature stem cuttings of *Holoptelea integrifolia* Planch.

3.4.5.1 Introduction

*Holoptelea integrifolia* Planch. Is being raised in the form of amenity plantations in north India especially in Haryana, Punjab and Delhi. There is natural variation in the vigour of the plant that gets expressed late in the life of species. CPTs have been marked for collection of seeds / fruits and also explants for root induction studies. Trees belonging to this species are a source of fodder and timber of some value. The leaf is a good fodder and can be repeatedly harvested during fodder scarcity period of the year. It is a tree that is pollinated by wind dilution of ‘elite’ traits is a distinct possibility when the tree out breeds. *Ex situ* conservation of CPTs can be done if explants could be asexually propagated, therefore, the experiment.

Objectives of the studies

i) To determine the affect of PGRs on rooting of mature stem cuttings to increase availability of good quality seedlings; and
ii) verify suitability of vegetatively propagated seedlings as possible alternatives to seed-origin seedlings; and

iii) *ex situ* collection of germplasm for raising clonal seed orchards (CSO).

### 3.4.5.2 Material

Stem cuttings from mature CPTs were collected during the month of May, those cuttings that were about 3 cm in diameter at the base; were one-two year old and vigorous in growth were preferred. Collection was so timed that the bud break was about to occur. Vegetative bud break occurs slightly late in the summer following a flush of reproductive bud break. The explants were surface sterilized and top portion sealed by dipping in to molten wax as explained elsewhere in the thesis. Every replication was set in either HDPE composite tray of forty cells; each of 110 ml capacity filled with vermiculite medium or sterilized Yamuna river sand.

### 3.4.5.3 a Experimental details

Stock solutions of IBA, NAA, Putrescine, Catechol and Boric Acid were prepared discussed in paragraph 3.2.5.2.a read with Table: 3.2 and note there under.

### 3.4.5.3 b Treatments of the cuttings.

Freshly cut stem cuttings were brought wrapped in moist gunny bags from the tree to location where experiment was to be set to prevent loss of endogenous moisture. Surface sterilization was done in four stages as explained earlier under paragraph 3.2.6.2.a. Cuttings thus prepared were treated to designed concentrations of PGRs by dipping lower 3-5 cm portion in to the solution for a period of 24 hours. These treated cuttings were planted either in sand beds (gray biotite mica rich Yamuna river sand) / composite HDPE root trainers containing horticultural grade 4-8 mm vermiculite. The set up was subjected to hourly irrigation by micro - sprinklers under an open to sky situation. Those cuttings with developed shoots and roots were transplanted in to beds or polythene bags containing garden soil after suitable hardening. Identity of the treatment was maintained using aluminum tags written over with stylus.

### 3.4.5.3 c Observations

All the morphological data were recorded in multiples of ten i.e., 10, 20, 30 and 40 days. To study the seasonal affect, rooting trials were conducted at three month intervals in October, January, April and July of year 1999 -2000. Subsequent to that first experiment was set during May 2000. Repeatability was verified by setting two more
experiments during 2001 and 2002; on the basis of observed growth behaviour in different pheno-phases of the plant learnt in the trials conducted earlier.

3.5 The monkey-jack (*Artocarpus lakoocha* Roxb.) barhal (Hindi), dehu (Punjabi)

3.5.1 Introduction

*Artocarpus lakoocha* Roxb. is a long lived, laticiferous, macrophyllus, monoecious, lofty, handsome, deciduous tree. It is indigenous to sub-Himalaya east of Kumaon and south India (Parker, 1950). It is cultivated in wet hilly locations of Punjab, also in Kangra district of Himachal Pradesh and vegetable gardens of Delhi state. This tree occurs in parts of old Ambala district especially under cultivation in Morni hills, now included in Panchkula district, number of mature trees within the area of study i.e., Morni hills were less than 50.

3.5.1a. Following major areas were studied

- Survey of base population in Morni hills in the Siwalik hill ranges of Haryana state. Identification of healthy phenotypic superior trees that bear large quantity of fruits in greater quantity, called as candidate plus trees (CPTs).
- Tree-wise (CPT-wise) collection of raw and ripe fruits that were used for germination studies.
- Raised tree-wise (CPT-wise) seedlings for carrying out plantations and further studies.
- Carried out clonal propagation (vegetative), of juvenile and root cuttings and mature stem cuttings, especially those obtained from CPTs.

3.5.2 Survey of base population

Complete enumeration of all the fruit bearing trees was done in Morni hills. Out of that 2 trees were found to be promising and their details were collected as given in table 3.

6. Growth and fruit production in trees situated along rain water drains is better as ecological requirements of the species are fully satisfied. In other places where water
Table: 3. 7 Geographic locations of CPTs selected

<table>
<thead>
<tr>
<th>Name of provenance</th>
<th>Geographic location</th>
<th>Size of base Population</th>
<th>No. of candidate plus trees selected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N. Latitude E. Longitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) Morni hills</td>
<td>30° 41” 77° 10”</td>
<td>43</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>43</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>
availability is less or root growth is obstructed because of rock strata, tree produces much less fruits. Under agroforestry situation tree produces more fruits but during warm part of the year canopy casts dense shade where paddy cultivation becomes almost impossible. Thus this species is half-compatible i.e., compatible for rising ‘rabi’ or winter crop of wheat when it is leafless. In discriminate extraction of fruits, short viability of seeds, initially slow growing seedlings, climate and herbivore damage are different factors that have led to lack of regeneration.

3.5.3 Studies on germination

3.5.3.1 Introduction

*Artocarpus lakoocha* Rox. is one of the lesser known tree species that naturally occurs in sub-tropical and deciduous forests of India. It is especially known for its fruits that are used in pickle making. It is a very important non-timber forest produce plant (NTFP). Natural regeneration was found to be almost negligible in area of study within Haryana state covering three districts especially in Morni hills of Panchkula district. Investigations were carried out to understand the reasons responsible for lack of natural regeneration and one of the steps was studies on germination.

3.5.3.1. a Material

*Artocarpus lakoocha*, Rox. seeds collected from mother trees collected as CPTs, bought from commercial sources and also other healthy trees.

3.5.3.1. b Objective of the experiment

Objective of the study was to determine the role of various factors effecting germination with a view to solve the problem of scanty natural regeneration.

3.5.3.1. c Experimental details

Treatment: i) Nature simulated conditions; experimental material used were ripe fruits that were shed and decay on the floor and seeds that were spit by fruit eating animals. Seed Source: Sector 21 Government Nursery, Chandigarh (1 tree), Kajar village (3 trees) and Sultanpur Jaloli (2 trees). Seeds were separated in to three size classes by ocular estimation of volume and also weight.

Control: No of seeds 80 and weight 48.5 g; weight of sample seed 0.61 g.
Large: No of seeds 80 and weight 66.0 g; weight of sample seed 0.83 g.
Medium: No of seeds 80 and weight 52.5 g; weight of sample seed 0.66 g.
Small: No of seeds 80 and weight 34.0 g; weight of sample seed 0.43 g.
All the morphological data were taken after 30, 60, 90, 120 and 150 days.

U.P Hill Commercial source 4 kg Rs 12.5 / kg.; nine fruits from top selection; 444 g per fruit; largest fruit size seen so far late during season; September 8th, 2003. No. of seeds 76

U.P Hill Commercial source 10 kg Rs 15/ kg.; 47 fruits from top selection; 213 g per fruit; medium type from north of Saharanpur seen late during season; September 13th, 2003. No. of seeds 50

Simulation of what happens in nature was done and ripe seeds were sown in three replications of 30 seeds each, during the month of June. Observations were recorded twice a month. Data regarding germination that commences during June and continues up to the end of September was recorded.

Treatment: ii) Artificial ripening condition; experimental material used about to ripe fruits. Fruits of CPTs were collected tree-wise and stored in open till they started to decay. After decay set in completely; the seeds were extracted from pulpy fruit and washed clean in running water. Surface fungal disinfection was done by 2 % water solution of emesan for 5 minutes. Surface was washed thrice in running water to remove residues of emesan. Subsequently seeds were steeped in 2 % solution of bavistin. Pretreated seeds were sown in a time series commencing from June third week up to September in 110 ml root trainers filled with sterile vermiculite. Above set up was subject to ‘open mist’ as explained in paragraph 3.2.3.3.a above. Mean maximum temperature during late May and June is 36 °C or - 6 ° Celsius and relative humidity varies between 45 - 65 %. Observations on germination and growth were recorded fortnightly in three replications with sample size of 30.

Treatment: iii) De-husked ripe seeds were sown in to garden soil filled in root trainers without any disinfection

Fully ripe fruits were de pulped manually seeds so separated were sown in to garden soil filled root trainers without any surface sterilization. They were exposed to micro-sprinkler irrigation. Observations were timed similar to treatment I. i)

Treatment: iv) Sowing of perianth covered seeds

A membranous perianth covers the seed in side the fleshy receptacle; often it is found dry and sticks to germinating seeds. To evaluate the protective role of this layer; it was retained as such and sown into soil by keeping all other conditions similar to I.iii treatment. Observations were timed similar to earlier named treatment.
Treatment: Control. An equal number of all the above types of seeds were sown in garden soil in rows. Observations were timed similar to other treatments.

3.5.4. Effect of PGRs on rooting and sprouting of juvenile stem cuttings of *Artocarpus lakoocha* Roxb.

3.5.4.1 Introduction

Near total extraction of fruits for commercial purposes and change in forest floor characters in the area of study and possibly in other areas within the range of distribution of the species has reduced natural regeneration. In Morni hills of Panchkula district hardly any established natural seedling was seen during survey conducted over four years. In the ‘transitional zone’ i.e., theoretically fringe areas of occurrence of a species, compared to ‘core’ areas, natural regeneration is always a problem. The reason is some of the essential requirements are not met to the same extent as in the core area. Degradation of habitat only accentuates the problem as has happened in the area of study. Alternatives are to be evolved for ensuring supply of selected seedlings so that the limiting factor of non availability of the most important input i.e., seed is overcome. In this direction vegetative propagation holds promise hence the studies.

3.5.4.2 Objectives of the studies

To determine the effect of PGRs on rooting of juvenile stem cuttings to increase availability of good quality seedlings; and verify suitability of asexually propagated seedlings as possible alternatives to seed-origin seedlings.

3.5.4.3 Material and methods

3.5.4.3. a Preparation of stock solution

Stock solutions of desired concentrations of IBA, NAA and Catechol were prepared in distilled water as explained in paragraph 3.2.5.2.a above.

3.5.4.3. b Preparation of the cuttings

The stem cuttings (explants) were taken during the month of April from nine month – old seedlings of *Artocarpus lakoocha*, Roxb. that were raised in a nursery using 15 x 22 cm polythene bags. At this stage every seedling is capable of giving only one cutting with a mean size of 12 cm; as the height of seedling is not more than 20 cm.

3.5.4.3. c Treatment of the cuttings

Freshly severed explants after surface sterilization (as explained elsewhere in the thesis) were treated with three concentrations viz., (i) 0.1 μ M (ii) 0.2 μ M and (iii) 0.3 μ
M each of IBA, NAA and Catechol by dipping lower 2 cm portions into water solution for a period of 24 hours i.e., long duration soaking. Other details of treatments are as per details given in paragraph 3.2.5.3 above. The sprouting response was observed after 10, 20, 30 and 40 days of plantation and rooting response at the end of 40 days but indications were drawn from sighting of roots in the lower portion of root - trainer.

3.5.5. Effect of PGRs on rooting and sprouting of mature stem cuttings of *Artocarpus lakoocha* Roxb.

3.5.5.1 Introduction

Fruit bearing commences from sixth year in *Artocarpus lakoocha* Roxb. and commercial bearing from about ten years. As the fruit is the commercial product if waiting period is reduced then economic appeal of the plant improves. Also rooted branches of elite trees produce true-to-type fruits making it possible to begin new lines of domesticated varieties.

3.5.5.2 Material

3.5.5.2. a Preparation of cuttings

The mature stem cuttings were taken from CPTs of *Artocarpus lakoocha* Roxb. shown in the Table: 3.6 particularly from Kajar village of Momi hills.

3.5.5.2. b Preparation of stock solution

Stock solutions of desired concentrations of IBA, NAA, putrescine and Catechol were prepared in distilled water. Other details of treatments are as per details given in paragraph 3.2.5.2.a above.

3.5.5.2. c Treatment of the cuttings

Freshly severed stem cuttings, after surface sterilization as explained elsewhere in the thesis, were treated with three concentrations each of IBA, NAA, Putrescene and Catechol by dipping lower 5 cm portions into the solution (long duration soaking) as follows (i) 0.1 mM (ii) 0.2 mM and (iii) 0.3 mM in water for a period of 24 hours. Cuttings were planted in to vermiculite medium filled in composite 110 ml capacity root trainers with 40 cells. Irrigation was provided by 'open misting' as explained in paragraph 3.2.3.3.a above.

3.5.6 Experiment to induct sprouting of tap root and root-shoot cuttings of *Artocarpus lakoocha* Roxb.

3.5.6.1 Introduction
Seeds of *Artocarpus lakoocha* Roxb. were found to be viable for less than a fortnight. Juvenile shoot cuttings sprout and root but indifferently; also the problem of non formation of tap-root quite often defeats the purpose of propagation. PGR induced rooting requires some one specially trained and dedicated to do that activity and failures were not taken kindly by the system. Thus a search was under taken to over come a difficult problem of vegetative propagation acceptable to the skilled gardener ‘the mali’.

### 3.5.6.2 Material

This experiment was conducted to study the sprouting and rooting behaviour of juvenile tap-root and root-shoot cuttings of *Artocarpus lakoocha* Roxb. during summer. If small sized plants are transplanted they find it difficult to survive under harsh field conditions. One of the ways to harden seedling was to increase duration of stay in the nursery. Root-shoot cutting is one year time advanced propagule that has juvenility, vigour and also required sturdiness. Physically it has shoot portion above the collar region that is up to 10 cm and tap-root portion two to three times the length of shoot. This is successfully used in other species also e.g., *Dalbergia sissoo, Acacia catechu* and the idea was successfully extended to species under study.

### 3.5.6.3 Preparation of the cuttings

The juvenile tap-root and root-shoot cuttings were taken from nursery raised seedlings that were 9 months and 21 months old for which seeds from CPTs of *Artocarpus lakoocha* Roxb. occurring in locations shown in the Table:3.7 particularly from Kajar village of Morni hills were used.

### 3.5.6.4 Preparation of stock solution

Stock solution of required concentration of putrescine were prepared in distilled water. Different concentrations for designed treatments were made by serial dilution technique. Weighed quantity of putrescine is directly dissolved in water and serially diluted there after to obtain required concentration. Treatment of the cuttings with distilled water served as control.

### 3.5.6.5 Treatment of cuttings

Freshly severed tap-root and root-shoot cuttings, after surface sterilization as explained elsewhere in the thesis, were treated with three concentrations each of Putrescine by dipping lower 5 cm portions into the solution (long duration soaking) as follows (i) 0.1 mM (ii) 0.2 mM and (iii) 0.3 mM in water for a period of 24 hours.
Cuttings were planted in to vermiculite medium filled in composite 110 ml capacity root trainers with 40 cells. Irrigation was provided by ‘open misting’.

3.5.7 Studies on air layering of mature *Artocarpus lakoocha* Roxb.

3.5.7.1 Introduction

*Ex situ* conservation of economically more valuable germplasm and also mass multiplication holds the key to making alternative indigenous tree species available to tree farmers. As the ‘elite’ trees are very few and far between their fruits are preferably bought by connoisseurs leading to decreased availability of seeds. Increasing their numbers by producing air layered plants was attempted to overcome above problem. Initially trials were laid out in all the four quarters of the year but later on the basis of experience gained it was restricted to early spring (February).

3.5.7.2 Plant material

Plant material that was used: mature trees selected as CPTs and other trees occurring naturally in Morni hills and Sultanpur village. Age of the trees varied between 40-80 years. Actual part used was 2-6 cm diameter branches both main and side branches were used. Age at the point of air-layer was less than five years and more often between 1-3 years.

3.5.7.2. a Preparation of the stock talc powder

Stock talc powder of IBA, NAA, and Boric Acid were prepared as detailed in paragraph 3.2.7.3. read with Table: 3.2 and note there under. Homogeneity of talc mixture was ensured by mixing in the slow speed electric mixer. All stock mixtures were stored in refrigerator using opaque non reactive (tapper ware) plastic containers with air tight lids. The mixtures of desired concentrations required for treatment were prepared by suitably diluting the stock mixtures with talc. Treatment of the layers with talc without any PGRs served as the control. As most of the trials made earlier had indicated that root initiation process was not fully accomplished in mature plants by using permutations and combinations as shown in treatments of juvenile air-layers some more treatments were also included for air-layering mature trees using various concentrations of IBA as indicated in the table.

3.5.7.3 Method of air layering

Same method as explained under relevant portion of *Terminalia chebula* Retz.in paragraph 3.2.9.3.b was done.