CHAPTER 6

Hardwickia binata Roxb.
Hardwickia binata Roxb., an endemic taxon to India, is a handsome deciduous tree, with graceful drooping branchlets. This tree yields an extremely hard, heavy and durable timber, known in the trade as “Anjan”. The wood is perhaps the hardest, heaviest in India. This tree can thrive in dry areas and even can withstand for prolonged drought. Inspite of its excellent characters as a reforestation crop in drought prone areas, this tree is not planted extensively due to the difficulty in getting enough seedlings, as the seeds are prone to pathogenic attack during maturation.

6.1 Taxonomy:

The genus Hardwickia of the family Caesalpiniaceae is represented by a single species Hardwickia binata Roxb.

6.2 Geographical Distribution:

Hardwickia binata is restricted in its distribution and found in the dry Savannah forests of the Deccan peninsula, Central India and parts of U.P. and Bihar (Anonymous, 1959).

6.3 Morphology:

Hardwickia binata is moderate to large sized tree, leafless for a short time or nearly evergreen, with drooping slender branchlets with greyish green coriaceous bifoliate leaves. This handsome deciduous tree attains a height of 36 m and a girth of 4.5 m with a clean, cylindrical bole. Bark is dark grey, rough with irregular vertical cracks exfoliating in narrow flakes (Fig. 40).
Leaves are alternate, bifoliate and the tree is leafless or nearly so for a short time towards the end of cold season. The new leaves tinged with red appear in April. In the hot weather the trees appear with feathery foliage, when most other species are leafless. Leaflets are sessile, entire, obliquely ovate and coriaceous.

Small, pale yellowish green flowers in axillary and terminal lax panicled racemes appear from July to September. The pod is flat and samaroid, 5 - 7.5 cm / 1 - 1.5 cm, oblong lanceolate, coriaceous, narrowed at both ends, with parallel longitudinal veins, containing one seed near the apex (Fig. 41). The seed is exalbuminous, flat, averaging 2 X 0.75 cm, sub-reniform, pointed at one end and rounded at the other, with a fairly hard testa.

Wood is extremely hard. The sapwood is small and white. The heart wood is dark reddish brown streaked with purple close grained dull with oily feel, irregularly interlocked, grained and coarse-textured.

6.4 Climate:

6.4.1 Soil:

*Hardwickia binata* thrives in a dry climate and is capable of growing on dry shallow soil and rocky ground. Its best development is attained in porous sandy loams and reddish gravelly sand overlying sand stone, conglomerate granites and schists, with an overlying soil of sandy loam. The tree frequently attains a large size, even though the overlying soil may not be deep, since the tap root has a wonderful capacity for making its way through fissure in solid rock (Fig. 42).

6.4.2 Light and Temperature:

The tree is capable of standing a certain amount of shade in youth and even requires shelter in its young stages. It may be classified as a moderate light demander or partial shade bearer. In its natural habitat, the maximum shade temperature varies from 40° to 45°C and the minimum temperature varies from 1° to 10°C (Troup, 1921).
6.4.3 Rainfall:

*Hardwickia binata* resists the dry climate characterized by a prolonged period of drought, scanty to moderate rainfall and intense heat during hot season. The normal rainfall varies from 25.4 cm to 182.4 cm. It appears to survive best with a rainfall of 50.8 cm to 100 cm (Troup, 1921).

6.5 Diseases and Pests:

The information regarding diseases and pests that attack during mature stage is lacking. Soni et al., 1989 reported pathogenic attack at maturation of seeds which ultimately lead to death. Troup (1921) reported damping off, rot and die back of seedlings when grown on moist weedy soil.

6.6 Economic Importance:

Anjan wood is hard, heavy, durable resistant to rot and white ants. It is largely used for naves of cart wheels, oil mills, ploughs, cold crushers, posts, beams, mine props, bridges, wells, pontoons, oars and parquet floors. It is also used for carving, turning and ornamental work. It is suitable for bench screws, lathe chucks, tool handles, sheaves of rope blocks, railway keys, tent pegs and brake blocks.

The bark yields a red-brown fibre used for well ropes and other agricultural purposes. The leaves are used as cattle fodder and manure. The tree is often pollarded not only for the fodder and manure supplied by the leaves, but also for the fibre obtained from the young branches (Anonymous, 1959).

6.7 Conventional Methods of Propagation:

Natural regeneration is through seeds and sometimes it reproduces by root suckers. There are several investigations regarding the effect of seed size on germination, drought, frost, damp, grasses and weeds on early seedling mortality (Troup, 1921). Fresh ripe seeds have a high
percentage of fertility but it loses its viability within a small period of time. In its natural habitat, the seedlings are very sensitive to drought and the seedlings are prone to death. This fact has been emphasised again and again by different observers, and it may be said without question that the great mortality is noticeable among the numerous seedlings which appear after a good seed year is due to drought. If once the seedling happen to establish for a year or so it withstands for a longer period of drought and it is an excellent drought hardy species.

Seedlings are so sensitive to excessive moisture and thick cotyledons are immediately attacked by rot and thus the young seedlings will be destroyed in a single night. Careful removal of grasses without damaging the root or seedlings grown on soil clear of grasses and weeds may show good growth with less mortality.

Though this tree can be reproduced by root suckers in nature, the percentage of root suckers is low and Ram Prasad et al., (1992) reported that rooting of the cuttings was not successful and placed this tree under very difficult to root category.

6.8 Tissue Culture Studies:

*Hardwickia binata* Roxb., an excellent reforestation plant with its highly resistable characters to withstand prolonged drought, has a major drawback for its natural regeneration both via seeds and rooted cuttings. To supersede these obstacles and to provide an efficient method for rapid multiplication an attempt has been made to propagate this tree species through tissue culture technology. Detailed methodology adopted for establishing *in vitro* cultures were discussed in general methodology and specific methods are given in respective chapters. Most of the methods followed are similar to Red sanders, hence, only a brief account of methodology is mentioned.

6.8.1 Material:

Aseptically grown seedling explants and juvenile mature tree explants formed the source for *in vitro* cultures.
Mature dried pods were collected from various provinances viz., Andhra Pradesh Forest department, Anantapur; Muchukota reserve forest and S.K. University campus, Anantapur.

To raise aseptically germinated seedlings initially the germinability of seeds under natural conditions was determined. Per cent frequency of germination of seeds procured from various localities was examined, and the germination counts were compared and tabulated (Table-32). Seeds obtained from Muchukota reserve forest and S.K.U. campus exhibited high per cent frequency of germination in Petri plates. However, plant per cent in earthen pots was very low. An interesting observation in seeds germinated in Petri plates was, the seed coat leachates were too high, the radicle after two days turned black and growth was inhibited. Replacing filter paper every day, the growth of seedlings retained normally. This indicated that seed coat leachates were inhibitory to the growth of seedlings.

**Table-32**

<table>
<thead>
<tr>
<th>Place of Collection</th>
<th>% of Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andhra Pradesh Forest Department, Anantapur.</td>
<td>40</td>
</tr>
<tr>
<td>Sri Krishnadevaraya University Campus</td>
<td></td>
</tr>
<tr>
<td>Anantapur, A.P</td>
<td>60</td>
</tr>
<tr>
<td>Muchukota Reserve Forest, Anantapur, A.P</td>
<td>60</td>
</tr>
</tbody>
</table>

The seeds are generally damaged by insects and they loose viability within 6 months. The percentage of germination is more in freshly collected seeds and it decreased after a month. Seeds are therefore stored after treating them with a heavy dose of insecticide.

**6.8.2 Aseptic Germination:**

After separating the seed from pod by hand, they were treated with 100 PPM ascorbic acid for 5-10 minutes, then washed thoroughly with distilled water. Routine sterilization procedures were followed before inoculating onto germination media (same as used for Red Sanders). Inspite of treating seeds with various antioxidants (PVP, AC), overnight soaking and
cold treatment, copious amounts of seed coat leachates accumulated in the germination media and they inhibited the growth of radicle. An attempt was also made to culture the seeds in sterile culture vessels with tap water supported by filter paper bridges. Since there was no excess water in touch with seed coat, leachates did not come out and the radicles emerged and growth was good without any inhibition (Fig. 43a). Sixty per cent germination was attained by inoculating the disinfested healthy seeds. Within 10 days, the seedlings attained 5-6 cms with few nodes. Explants from 15 day old aseptically germinated seedlings were excised and used for establishing in vitro cultures.

**6.9 Protocols for Organogenesis:**

**6.9.1 Explant Evaluation:**

The choice of explant plays a critical role and it is a primary step to standardize a perfect *in vitro* regeneration system for mass multiplication. Various seedling explants were cultured on B₅ medium supplemented with 2 mg/l BAP to find out the best explant (Table-33).

<table>
<thead>
<tr>
<th>Explant</th>
<th>Per cent of explants responding</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>60</td>
<td>Brown coloured callus</td>
</tr>
<tr>
<td>Mesocotyl</td>
<td>0</td>
<td>2-3 Shoot buds</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>15</td>
<td>Very slight callus initiation</td>
</tr>
<tr>
<td>Shoot tip</td>
<td>0</td>
<td>1-2 Shoots</td>
</tr>
<tr>
<td>Nodal</td>
<td>0</td>
<td>2 Shoot buds</td>
</tr>
<tr>
<td>Leaf</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td>Inter nodal</td>
<td>5</td>
<td>Callus</td>
</tr>
</tbody>
</table>

Data represent average of 20 replicates.
The cultured explants excepting root and leaf resumed their growth either by proliferating callus, shoot buds or elongating the pre-existing meristems (Fig. 43b). Mesocotyls nodes and shoot tips proliferated shoot buds with varying frequencies. Multiple shoot regeneration was not observed except 2-3 shoot buds per mesocotyl explant were common (Fig. 44a). However, only single shoot elongated suppressing the growth of the other buds after one sub-culture (Fig. 44b). The induction of callus was also meagre in cotyledons and the explants remained green for several sub-cultures.

6.9.2 Media Evaluation:

Mesocotyl, shoot tip and nodal fragments were implanted on three different media and results are quantified after one sub-culture by visual observations (Table-34).

<table>
<thead>
<tr>
<th>Medium</th>
<th>Explant</th>
<th>Per cent frequency of response</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>B5</td>
<td>Shoot tip</td>
<td>40</td>
<td>Elongation</td>
</tr>
<tr>
<td></td>
<td>Nodal</td>
<td>40</td>
<td>Elongation</td>
</tr>
<tr>
<td></td>
<td>Mesocotyl</td>
<td>50</td>
<td>3 Shoot buds, necrosis</td>
</tr>
<tr>
<td></td>
<td>Shoot tip</td>
<td>60</td>
<td>Elongation</td>
</tr>
<tr>
<td></td>
<td>Nodal</td>
<td>50</td>
<td>1-2 Shoot buds</td>
</tr>
<tr>
<td></td>
<td>Mesocotyl</td>
<td>80</td>
<td>2-3 Shoot buds, but single shoot elongated</td>
</tr>
<tr>
<td>MS</td>
<td>Shoot tip</td>
<td>40</td>
<td>Elongation</td>
</tr>
<tr>
<td></td>
<td>Nodal</td>
<td>30</td>
<td>Elongation</td>
</tr>
<tr>
<td></td>
<td>Mesocotyl</td>
<td>70</td>
<td>Single shoot bud</td>
</tr>
<tr>
<td>WP</td>
<td>Shoot tip</td>
<td>40</td>
<td>Elongation</td>
</tr>
<tr>
<td></td>
<td>Nodal</td>
<td>30</td>
<td>Elongation</td>
</tr>
<tr>
<td></td>
<td>Mesocotyl</td>
<td>70</td>
<td>Single shoot bud</td>
</tr>
</tbody>
</table>

Data represent average of 20 replicates.

The highest frequency of shoot bud proliferation was observed in cultures established on MS medium. Both MS medium and B5 medium induced 2-3 shoot buds per explant with varying per cent frequencies. Shoot buds after elongation showed necrosis and percent of...
necrosis was very high on B5 medium, whereas in MS medium single healthy shoot elongated suppressing the other two shoot buds without any sign of elongation (Fig. 45). Shoot tips and nodal explants elongated on all the three media without any morphological variation. Considering the healthy, quick and high per cent shoot bud regeneration, MS medium was preferred to find out the various other factors that control morphogenesis.

6.9.3 Hormone evaluation:

To trigger the inherent morphogenetic capacity in shoot tips, nodal and mesocotyl explants, various hormones individually and in combinations were employed at different concentrations. The results are recorded after every 7 days and summarised.

6.9.4 Effect of BAP and KN:

Mesocotyls:

Initiation of shoot buds was observed from 10th day onwards. Of the two cytokinins tested, BAP was found to be more effective in terms of per cent efficiency (Table-35). The number of shoot buds regenerated was not encouraging at the concentrations of KN and BAP tried. The proliferated shoot buds after 2 sub-cultures elongated and the morphological nature of the shoots was not satisfactory on KN containing media. The average length of shoots steadily increased with increase in concentrations of both BAP and KN (Figs. 46a and 46b).

### TABLE - 35

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Conc. mg/l</th>
<th>Per cent of response</th>
<th>Length of shoots Mean + S.E</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>1</td>
<td>20</td>
<td>0.06 ± 0.050</td>
<td>1-2 Shoot buds</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>80</td>
<td>1.96 ± 0.027</td>
<td>2-3 Shoot buds</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>90</td>
<td>3.15 ± 0.044</td>
<td>2-3 Shoot buds</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>75</td>
<td>3.60 ± 0.025</td>
<td>Single shoot</td>
</tr>
</tbody>
</table>
Shoot Tips and Nodal Explants:

Most of the explants cultured in vitro proliferated 2 shoot buds per explant (Tables-36 and 37). However, one of the shoot buds necrosed and a solitary shoot bud elongated. Except for one or two concentrations (2 and 3 mg/l) all other concentrations of BAP and KN exhibited single bud proliferation and hence may be unsuitable for mass multiplication (Fig. 46c).

Table-36

Effect of different concentrations of BAP and KN on morphogenetic response of nodal explants of *Hardwickia binata* cultured on MS medium

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Conc. mg/l</th>
<th>Per cent of response</th>
<th>Length of shoots Mean ± S.E</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>1</td>
<td>30</td>
<td>1.6 ± 0.033</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50</td>
<td>2.3 ± 0.1</td>
<td>2 Shoot buds</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>60</td>
<td>2.12 ± 0.033</td>
<td>1-2 Shoots</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>65</td>
<td>3.18 ± 0.058</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>60</td>
<td>3.68 ± 0.036</td>
<td>Single shoot</td>
</tr>
<tr>
<td>KN</td>
<td>1</td>
<td>20</td>
<td>1.16 ± 0.05</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>45</td>
<td>1.625 ± 0.076</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>60</td>
<td>3.1 ± 0.033</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>65</td>
<td>3.2 ± 0.042</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>70</td>
<td>3.57 ± 0.03</td>
<td>Single shoot</td>
</tr>
</tbody>
</table>
Fig. 40 Bark

Fig. 41 Pods and Seeds

Fig. 42 Seedling on Rocky Ground
Fig. 43a Aseptic Seedling

Fig. 43b Morphogenetic responses
Fig. 44 Bud Proliferation

Fig. 45 Morphogenetic responses of Mesocotyl
Fig. 46a Effect of BAP on Mesocotyl

Fig. 46b Effect of KN on Mesocotyl

Fig. 46c Nodal explant - Proliferation of single shoot
Table-37

Effect of different concentrations of BAP and KN on morphogenetic response 
shoot tips of *Hardwickia binata* on MS medium

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Conc. mg/l</th>
<th>Per cent of response</th>
<th>Length of shoots Mean ± S.E</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>1</td>
<td>30</td>
<td>1.23 ± 0.083</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>55</td>
<td>2.14 ± 0.037</td>
<td>1-2 Shoot buds</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>65</td>
<td>3.16 ± 0.031</td>
<td>1-2 Shoot buds</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>70</td>
<td>3.07 ± 0.018</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>60</td>
<td>4.22 ± 0.057</td>
<td>Single shoot</td>
</tr>
<tr>
<td>KN</td>
<td>1</td>
<td>25</td>
<td>1.33 ± 0.050</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>55</td>
<td>2.18 ± 0.051</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>60</td>
<td>3.15 ± 0.036</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60</td>
<td>3.23 ± 0.039</td>
<td>Single shoot</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>75</td>
<td>5.30 ± 0.053</td>
<td>Single shoot</td>
</tr>
</tbody>
</table>

Data represent average of 20 replicates.

6.9.5 Combination of BAP and KN:

As the number of shoot buds proliferated per each explant was not encouraging on medium supplemented with individual cytokinins, an attempt was made to study the effect of cytokinins in combination. Shoot bud proliferation was 80%, when BAP and KN were supplemented at 2 mg/l each. Though 2-3 shoot buds were observed after 10-12 days, two buds necrosed and finally single shoot bud elongated. However, the length of shoots and morphological nature of leaves varied with the concentrations and combinations of hormone used (Fig. 47 and Table-38).
Effect of different hormonal combinations on shoot bud regeneration in mesocotyl explants of *Hardwickia binata*

<table>
<thead>
<tr>
<th>Hormone (mg/l)</th>
<th>% of Shoot bud regeneration</th>
<th>Average length (cm)</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>KN 1 BAP 1</td>
<td>50</td>
<td>1.540 _+_0.065</td>
<td>2-3 Shoot buds, necrosis</td>
</tr>
<tr>
<td>2 1</td>
<td>75</td>
<td>2.157 _+_0.044</td>
<td>1-2 Shoot buds</td>
</tr>
<tr>
<td>2 2</td>
<td>80</td>
<td>3.237 _+_0.048</td>
<td>2-3 Shoot buds</td>
</tr>
<tr>
<td>1 2</td>
<td>70</td>
<td>1.228 _+_0.056</td>
<td>1-2 Shoot buds</td>
</tr>
</tbody>
</table>

Data represent average of 20 replicates.

**6.9.6 Auxins and cytokinin combinations:**

The effect of BAP along with varied concentrations of NAA, IAA, IBA and GA3 was tested and the results are summarised in Table-39. Both per cent frequencies and number of shoot buds proliferated were not satisfactory (Fig. 48, 49 and 50). Mesocotyls cultured on BAP in combination with IBA exhibited rooting (Fig. 51). The explants cultured on GA3 containing media did not respond and hence omitted from the Table-39. Thus, inspite of the use of many hormonal combinations and permutations, *Hardwickia binata* did not respond well and seemed to be recalcitrant.

<table>
<thead>
<tr>
<th>Hormone (mg/l)</th>
<th>% of Shoot bud regeneration</th>
<th>Average length (cm)</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP 3 NAA 0.1</td>
<td>40</td>
<td>0.63 _+_0.076</td>
<td>Single shoot</td>
</tr>
<tr>
<td>3 0.5</td>
<td>50</td>
<td>0.80 _+_0.077</td>
<td>1-2 Shoots</td>
</tr>
<tr>
<td>2 1.0</td>
<td>40</td>
<td>2.40 _+_0.090</td>
<td>Single shoot</td>
</tr>
<tr>
<td>1 1.0</td>
<td>40</td>
<td>2.56 _+_0.033</td>
<td>Single shoot, not healthy</td>
</tr>
</tbody>
</table>
6.9.7 Coconut milk:

Owing to the importance of coconut milk on morphogenesis, the mesocotyls were cultured on MS medium supplemented with varying concentrations of coconut water (5-20%). Percentage efficiency of shoot bud regeneration decreased as the concentration of coconut milk increased in the medium. At 10% level, abnormal internodal length was noticed. Rooting was also observed on media with 5% coconut water (Table-40 and Figs. 52a and 52b).

<table>
<thead>
<tr>
<th>Coconut milk (%)</th>
<th>% of cultures responded</th>
<th>Average length ± S.E</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>75</td>
<td>2.20 ± 0.062</td>
<td>Single healthy shoot with rooting</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>1.92 ± 0.067</td>
<td>1-2 Shoots</td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>1.75 ± 0.12</td>
<td>Single shoot</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>1.00 ± 0.00</td>
<td>Single shoot</td>
</tr>
</tbody>
</table>

Data represent average of 20 replicates.
6.9.8 Carbon Sources:

Sucrose was found to be superior for shoot bud regeneration followed by cane sugar and fructose and glucose was the least effective. The average length of elongated shoots was better in sucrose and cane sugar when compared to other carbon sources (Table-41 and Fig. 53).

<table>
<thead>
<tr>
<th>Carbon source (2%)</th>
<th>% of cultures responded</th>
<th>Average length ± S.E</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>80</td>
<td>2.59 ± 0.019</td>
<td>2 Shoots</td>
</tr>
<tr>
<td>Glucose</td>
<td>40</td>
<td>0.92 ± 0.029</td>
<td>Single shoot</td>
</tr>
<tr>
<td>Fructose</td>
<td>50</td>
<td>1.30 ± 0.034</td>
<td>Single shoot</td>
</tr>
<tr>
<td>Cane sugar</td>
<td>55</td>
<td>2.24 ± 0.040</td>
<td>Single shoot</td>
</tr>
</tbody>
</table>

Data represent average of 20 replicates.

6.10 Callus Cultures:

Auxins such as 2,4-D, 2,4-5T, 2,4-5TP, IAA, NAA, IBA when used at different concentrations and combinations were not effective in proliferating healthy callus. Explants either remained green without any response or initiated little callus at the cut ends which turned brown within 2-3 days (Fig. 53 a).

6.11 Rooting:

The regenerated shoots after elongation were excised and subjected to rooting like that of Pterocarpus santalinus (Chapter IV). Liquid medium was not suitable for Hardwickia binata and rooting was inhibited in liquid media supported by filter paper bridges (Fig. 54a). However, rooting could be induced on solid agar medium (Fig. 54b). To optimize the hormonal concentration, IBA, IAA and NAA at varied concentrations were employed to 1/2 strength MS.
Fig. 47 Effect of BAP + KN (mg/l)

Fig. 48 Effect of BAP+NAA (mg/l)

Fig. 49 Effect of BAP + IAA (mg/l)
Fig. 50 Effect of BAP + IBA (mg/l)

Fig. 51 Rooting on BAP + IBA (mg/l)
Fig. 52a Effect of Coconut milk (%)

Fig. 52b Rooting at 5% C.M
Fig. 53 Effect of Carbon sources

Fig. 54 Dark Brown Callus.
The results obtained revealed that IBA was better, when compared to the other two auxins. Rooting response was 45%, from the regenerated shoots when implanted on the medium supplemented with 4 mg/l IBA. Three to four sub-cultures onto the same medium fortified with high concentrations of auxins was essential to induce good rooting in *Hardwickia binata*. Healthy tap roots with root hairs were regenerated from *in vitro* grown shoots when cultured on media supplemented with either IBA, or IAA or NAA at 4 mg/l. The per cent frequencies of response however, varied (Fig.55).

**Table-42**

**Effect of different auxins at varied concentrations on rooting response of *Hardwickia binata***

<table>
<thead>
<tr>
<th>Medium + Hormone</th>
<th>Concentration (mg/l)</th>
<th>% Frequency</th>
<th>Nature of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2 MS + IBA</td>
<td>0.1</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>15</td>
<td>Little root initiation</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>40</td>
<td>Root hairs</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>45</td>
<td>Tap root with root hairs</td>
</tr>
<tr>
<td>1/2 MS + NAA</td>
<td>0.1</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>5</td>
<td>Root hairs</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>25</td>
<td>Root hairs</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>35</td>
<td>Tap root with root hairs</td>
</tr>
<tr>
<td>1/2 MS + IAA</td>
<td>0.1</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0</td>
<td>No response</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>30</td>
<td>Single root</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>35</td>
<td>Single stout root</td>
</tr>
</tbody>
</table>

Data represent average of 20 replicates.

Attempts to establish suspension cultures and long-term cultures failed. Nodal and shoot tip cultures yielded a limited success with elongation.
6.12 Acclimatization:

Rooted plantlets were carefully removed from the culture vials, routine procedures presented in general methodology were followed to acclimatize the plantlets. Limited success has been achieved and the trials are under way to improve the frequency of plantlet survival in soil (Fig. 56).

6.13 Mature Tree Explants:

Nodal and shoot tip explants procured from juvenile twigs of mature trees were cultured for their morphogenetic potentiality. Though various hormonal treatments were tried, all the explants invariably induced dark brown callus (Fig. 57) but not shoots.
Fig. 54a Liquid medium supported with filter paper bridge

Fig. 54b Rooting on solid medium

Fig. 55 Rooting on different auxins
Flow chart of the established protocol for rapid multiplication of *Hardwickia binata*

Aseptic germination of seeds (60% in culture vessels with tap water and filter papers) 

15 days

Aseptic seedling (5-6 cm) with 3-4 nodes. Explants cultured on MS + 2mg/l BAP + 0.05% AC

Mesocotyl Shoot tip Node Other explants

15 days 15 days 15 days 15 days

3 shoot buds (1 or 2 necrosed) elongation 2-3 shoot buds 1 elongated callus or no response

Subjected to further improvement by evaluating media and hormones

In spite of more than two hundred combinations this plant did not respond, except two to three shoot buds on elongation

Elongated shoots 4mg/1 IBA

15-20 days

Rooting

Total No of 12 Plantlets per a Single seedling can be obtained within 45 days of seed germination