Chapter VII

IN VITRO STUDIES WITH *Schefflera arboricola*

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Introduction:

Origin and distribution: It is a genus named in honour of JC Scheffler of Danzig, belonging to the family Araliaceae. It is a genus of about 150 species of tall shrubs or trees widely distributed in the tropics from Taiwan, Hong Kong, Phillipines, Malaya, Sumatra upto Newzealand and Australia.

General description: Leaves, various, mostly digitately compound, rarely simple and then usually mixed with compound leaves, very seldom double digitately compound; flowers 5-6 merous in large panicles, or racemes or umbellate, seldom in whorls; calyx-limb weakly developed, obscurely and shortly toothed or almost lacking; petal 5 or more (upto 15), mostly 5-6; stamens as many as the petals; Fruits globose to ovate or elongated, and angled.

Ornamental value: This genus includes glabrous or pubescent trees or shrubs or sometimes even climbing by means of switch-like branches, grown in the greenhouse. *Schefflera* is grown for their showy foliage. The glossy, variegated digitate, pamately compound leaves have high ornamental value. Their shrubby appearance, shade preference for their growth, make them perfect indoor plants.

Various species of *Schefflera*:

Schefflera arboricola: (Taiwan) **Hawailian Elf**, freely branching plants of dwarf habit, resembles as miniature *Brassaia* when young; wiry stems, flexible and becoming scandent to 15 cm across, arranged in a circle of 7 to 8 soft-leathery leaflets; inflorescence in erect, terminal cluster of orange-red to blackish berries; very charming in appearance and is a good decorative plant.

Schefflera delavayi: (China Yunnan, Honan), Spreading shrubs 4 m high, with large palmately compound leaves on long petiole, the thin - leathery ovate leaflets

mattle-brownish green, brown pubescent underneath, faintly toothed, or lobed at margins and with prominent midrib; long inflorescence with white flowers; resistant to cold.

Schefflera digitata: (New Zealand) **Seven fingers**, bush or small tree, 3-6m high, densely branching, sometimes growing epiphytic with thin leathery leaves, palmately compound, 5-10 foliate obovate leaflets, to 17 cm long, dull satiny green above, shiny light green beneath, densely ciliate and undulate at margins and with yellowish, depressed veins; greenish-yellow flowers in panicles, purple-black fruit berry, prefers cool locations.

Schefflera farinose: (Malaya, Sumatra, Java), very ornamental tree with shiny stems covered by scales; the leaves palmate-compound, 35 cm across, the green leathery leaflets covered with mealy wax, and beautifully arranged in a formal circle; small whitish flowers in terminal inflorescence consisting of numerous incurring spikes.

Schefflera octophylla: (Hong Kong), an **umbrella tree**, branching small evergreen tree which one can be seen growing abundantly in the forest near the peak station of the stem above Victoria; Palmate leaves with 8-9 sturdy leathery, shining green leaflets having smooth entire margins.

***Schefflera octophylla*: Starshine** (Philippines), Handsome ornamental plant found in 1977 by Ingwersen of Oceanside, California, growing epiphite on a tree in Mindanao. It is tree- like but becoming scandent with willowy, beige-brown stems to 6 m high; long wiry petiole, carry the palmately compound striking leaves, 30 cm or more across, consisting of usually 9 to 12 pendant, narrow lanceolate leaflets 12 to 25 cm long, deeply corrugated, the leathery texture and lustrous deep green but tending to defoliate at cool temperature. It needs staking when used as a decorative plant.

Schefflera venulosa: (Queens land, China, Indonesia, India), a **Starleaf**, branching tree with palmately compound leaves; the 7 – 8 stalked leaflets lanceolate when

young, obovate or elliptic in maturity, soft-leathery, semi-glossy on both sides, to 15 cm long; mature leaves entire, dark green, but lightly toothed in juvenile stage; inflorescence in panicles with whitish flowers followed by small red fruit.

***Schefflera venulosa* var Erythrostachys:** (Tropical Asia to Australia), a **Starleaf**, Scandent evergreen to 6 m high, inclined to become semi-climbing, forming adventitious roots; leaves alternate, palmate with 6 – 7 leaflets, oval or ovate, fleshy, to 15 cm long, yellowish-green; globular flower dark red, in dense cluster.

Schefflera volkenisii: (Tanzania, Uganda), great evergreen tree to 25 m high from mountains at 2,200-2,700 m, such as growing in the mist forest belt on Mt. Kilimanjaro at 2,700 m. Palmately compound leaves with 5-7 glossy-leathery leaflets to 15 cm long, on willing branches.

Commercial value of *Schefflera* are in making various domestic items, eaten as raw and also used for flavouring many items (Table-7.1). Some of the uses are as follows:

Table - 7.1: Commercial uses of certain species of *Schefflera*

PLANT SPECIES	LOCATION	USES	REFERENCE
Schefflera aromatica	Malaysia and Indonesia	Young leaves are aromatic and is eaten raw although little acidic and bitterish Roots are also used for flavouring food	Berkel, 1935; Anonymous, 1976
Schefflera elata	Nepal & China	Wood used for box-planking	Anonymous, 1994
Schefflera hypoluca	Khasi hills	Poultice of leaves applied to swelling	Anonymous, 1994
Schefflera impressa	Nepal and China	Leaves are a good fodder Leaves also yield gum	Anonymous, 1994
Schefflera racimosa	-	Wood used for small boxes and light packing material	Anonymous, 1994
Schefflera venulosa	India (West-Bengal)	Roots are mixed with rice and eaten to cure dropsy	Anonymous, 1976
Schefflera wallichiana	Malaysia	Wood may be used for making tea boxes	Anonymous, 1976

The species chosen here is *Schefflera arboricola.* Because of its immense ornamental value at decorative places and easy growth of plant has fetched this a high commercial value as indoor as well as garden pot plants. As the world continues to grow as a function of the increased use of foliage in super market bouquets and indoor plants the fresh cut foliage trade showed 3% increase in 1999, equivalent to $620 million. Thus it becomes inevitable to mass propagate this highly commercial indoor foliage plant.

Materials and methods:

Plant Material: Healthy and mature plants growing in the Botanical Garden of the M. S. University, Baroda were selected for micropropagation (Fig 23-a). Initially some mature branches were cut and discarded to allow the juvenile shoots to appear and grow at the cut ends. The juvenile shoots with six to eight nodes were collected and their cut ends were immediately dipped in water. The axillary nodes were cut individually and exposed from juvenile shoots.

Sterilization Of Explants: Nodal explants were first washed under running tap-water and then treated with mild detergent - Teepol (1%; v/v) for 4-5 min and again kept under tap water. This was followed by pre-treatment of explants with a solution containing (0.05%; w\v) Bavstin and (0.5 %; w\v) Rifampicin and kept on the shaker (100 rpm) for 2 –3 hr followed by thorough washing with distilled water (3 times). Lastly the explants were treated with sterilant (0.1%;w\v) $HgCl_2$ for 5-6 min in the laminar flow and subsequently washing 3-4 times with sterile distilled water to remove the traces of $HgCl_2$

Initiation Of Cultures: For bud-break induction and growth of shoot, cytokinins KN and BA concentrations, as well as BA in combination with auxins IAA / NAA on MS medium were used. For multiple shoot induction various concentrations of cytokinins BA and KN alone and BA with KN in combination were used. Auxins NAA / IAA / IBA were tried in the rooting media. Acclimatization and hardening of plantlets were carried out in the culture room.

Fig 23: (a) *Schefflera arboricola plant* growing in the Botanical garden of M S University of Baroda-campus.


a

Results and discussions:

Contamination in cultures: It is a known fact that loss of cultures in *in vitro* propagation is mainly due to bacterial (Wainwright & England, 1978; Mathias et al, 1978) and fungal (Enjalric et al 1988) contaminants. Normally, exogenous microorganisms are effectively eliminated by using surface sterilants (Bonga, 1982). Internal contamination however is very difficult to eliminate and is a serious problem with explants of field grown plants (Von Arnold and Elikson 1986, Duhem et al 1998). Moreover, in explants of field grown ornamentals, contaminants are reported to make sudden appearance at later stages of growth *in vitro* (George and Sherrington, 1984). In *Schefflera arboricola,* the pre-treatment of explants with Bavistin and Rifampcin resulted in senesence of nodal explants. There was hardly any fungal contamination noted in the cultures after the treatment. Thus combined treatment of Bavistin (0.05%; w/v) and Rifampcin (0.5 %; w\v) did have a profound effect in reducing the contamination. There was a persistent occurrence of bacterial contamination even after strong sterilization treatments with $HgCl_2$ (0.1 %; w/v) for 5-6 minutes. The contamination free cultures of nodal explants were established on MS basal solid medium for about a week and then transferred to the MS medium containing BA and KN for bud break (Fig 24-a).

Axillary sprouting: The most common applied technique for micropropagation of ornamentals is the single node culture method resulting in the production of shoots and regeneration of roots on these shoots. The technique exploits the normal ontogenic route of branch development by lateral (axillary) meristems. As an axillary bud or apical bud grows into an elongated shoot, single nodes with respective dormant bud are subcultured for further multiplication. The rate of shoot multiplication in such cases depends on the number of nodal cuttings that can be excised from the shoot at the end of each passage.

This method is becoming increasingly popular for clonal propagation of plants because the organized shoot apices are least susceptible to genotypic changes under culture conditions. Shoot tip culture and axillary node culture is the most common of *in vitro*

propagation for many ornamentals. eg. *Jasminum officinale* (Bhattacharya & Bhattacharya, 1997), *Lavandula lalifolia* (Sanchez & Carmincalvo, 1996), *Chrysanthemum* (Jaacov & Langhans, 1972) etc.

Bud break response of axillary buds was visible from the 12th day onwards (Fig 24-b) on MS medium containing cytokinins BA and KN individually. Although the maximum percentage(75 % ± 3.2) of bud sprouting was achieved in BA (2.21 μM). However, for the growth of the shoots, comparing both the cytokinins, KN proved to be better than BA with average number of leaves/plant 4.5 ± 0.3 (Fig 24-c) and 1.1 ± 0.1 cm petiole length at 4.64 μM concentration (Table-7.2). BA on the other hand produced maximum 4 leaves / shoot with same concentration. The interesting point noted was that shoots produced in both cytokinins were found to be healthy and sturdy after 15 to 20 days. In some plant species lower concentrations of BA (1.7 μM) have been shown to inhibit shoot development and to be deleterious to shoot growth and quality (Welander, 1985; Drew and Smith, 1986). Depending on the species growing *in vitro*, BA can be either efficiently translocated into the upper parts of shoots or active mainly in the basal parts of the explant shoots (Biondi et al, 1984; Nordstrom and Eliasson 1986.)

In many of the micropropagation protocols cytokinin BA was found to initiate optimum results as in *Gmelina arborea* (Kannan and Jasrai, 1996). In *Isoplexis canariensis* shoot proliferation in liquid medium generally increased with BA concentration (Arrebola et al, 1997).

On subjecting the data to Anova test, there was a significant difference in the bud break response of the two cytokines used KN and BA.

Fig 24: Establishment and growth of axillary buds of *Schefflera arboricola.*

(a) Established bud on MS solid medium after 12 days of incubation.

(b) Extension growth of the axillary bud on MS solid medium containing BA (2.21 μM) after 20 days of incubation.

(c) Elongation growth of shoots on MS solid medium supplemented with KN (4.64 μM)

In figures, the bar = 1 cm

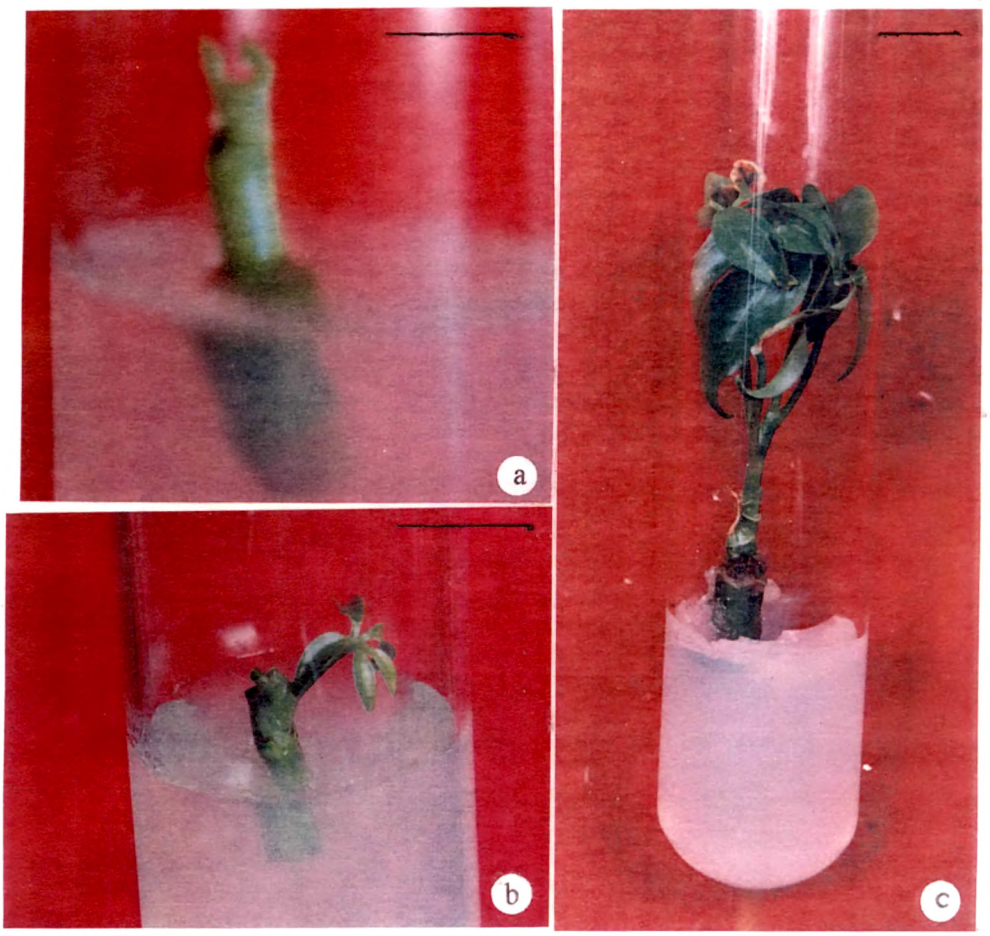
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Table-7.2: Bud sprouting response of nodal explants and Growth under different concentrations of cytokinins (KN/BA) in *Schefflera arboricola* after 15 days of incubation

CYTOKININS (μM)	BUD SPROUTING (%)* MEAN ± S.E.	NUMBER OF LEAVES/ SHOOT MEAN ± S.E.	PETIOLE LENGTH (cm)* MEAN ± S.E.
Basal medium	-	-	-
KN 0.46	-	-	-
1.16	18.3 ± 1.8	3 ± 0	1.0 ± 0.1
2.32	45 ± 3.8	4.5 ± 0.3	0.8 ± 0.1
4.64	58.2 ± 2.3	4.5 ± 0.3	1.1 ± 0.1
9.29	37.4 ± 1.8	2.5 ± 0.3	1 ± 0.1
BA 0.44	22 ± 3.5	0.5 ± 0.3	0.1 ± 0.07
1.10	45 ± 1.6	2 ± 0	0.6 ± 0.1
2.21	75 ± 3.2	3.5 ± 0.3	0.6 ± 0.1
4.43	66 ± 3.6	4 ± 0.7	0.9 ± 0.1
8.87	57 ± 4.2	3.5 ± 0.3	0.9 ± 0.1

± S.E. – Standard error
*Values are the percentage means of 3 independent experiments.

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	SIGNIFICANT
Between groups	4	2.6267	.6567	3.3448	.0119**
Within groups	145	28.4667	.1963		
Total	149	31.0933			

*The data was subjected to ANOVA at $p \leq 0.05$ significance
** Significant at $p \leq 0.05$

The effect of different auxins (IAA / NAA) in combination with cytokinin BA (2.21μM) on MS medium for bud initiation at all concentrations tried was poor (Table-7.3) compared to the cytokinins (BA / KN) used alone for bud sprouting (Table-7.2). The maximum response (50 ± 4.7%) was observed on MS medium containing IAA

(5.70 μM) with BA (2.21 μM). Increase in the level of IAA in the medium actually increased the sprouting, but after a certain level further increase in concentration showed a declining trend. Hence as a result auxins were not opted for the bud initiation.

Table - 7.3: Influence of Auxins (IAA / NAA) with cytokinin BA on bud sprouting in *Schefflera arboricola* after 15 days of incubation

GROWTH REGULATORS (μM)		BUD SPROUTING (%)* MEAN ± S.E.
Basal medium		10 ± 0
NAA	BA	
1.34	2.21	34.4 ± 2.4
2.63	2.21	41.6 ± 6.8
5.37	2.21	43.4 ± 2.7
10.74	2.21	27.1 ± 5.2
IAA		
1.42	2.21	13.3 ± 7.2
2.85	2.21	22.4 ± 3.4
5.70	2.21	50 ± 4.7
11.41	2.21	40.5 ± 0.4

± S.E. – Standard error
*Values are the percentage means of 3 independent experiments.

Multiple shoot induction: The well grown *in vitro* shoots (2 to 4 cm) were excised and subcultured as single explants for multiple shoot induction to reduce contamination in cultures. The multiplication medium was supplemented with various concentrations of BA and KN in combination and BA alone (Table 6.4). For multiple shoot induction, though all the concentrations of BA, KN and BA in combination with KN on MS medium responded after 15 days of incubation but higher level of BA (8.87 μM) proved to be the most suitable concentration which gave 67 ± 3.2% response (Fig

25-a), in *in vitro* explants for multiple shoots with 5.6 ± 1.5 number of multiple shoots (Fig 25-b). KN was found to be effective than BA for length of multiple shoots, whereas, combination of BA & KN together could not have a profound effect on this aspect. Thus the synergistic effect of both the cytokinins BA and KN could not help for multiple shoot initiation and development. However, a synergistic effect of KN and BA has been reported for multiple shoot induction in the family verbenaceae (Gupta et al, 1980; 1991; Devi et al, 1994). Growth and elongation of multiple shoots was observed on MS basal solid medium without any growth regulators in around 2 weeks (Fig 25-c).

The nodal explants were used, from *in vitro* grown shoots, to reduce the level of contamination in the cultures. In blue honey suckle (*Lonicera caerulea*) BA alone, strongly affected proliferation and growth of the shoots (Karhu, 1997). BA has also been used to micropropagate different Lonicera species (Bonnour et al, 1988; Laine et al, 1990; Ochatt, 1991). On the other hand D'silva and D'souza (1992) found that a high concentration of BA (22.2 μM) was required for the initiation of maximum number of buds from the cotyledonary nodes of cashew nut. Similarly BA was best suited for the further growth of multiples in *schefflera* upto 2-4 cm as compared to KN (Table-7.4).

Root Induction: Well-grown (3 to 5 cm) *in vitro* shoots were transferred for root induction medium with auxins (IAA, NAA and IBA) (Table-7.5). The shoots were noticed with maximum 2-3 roots after 15 days of incubation, on MS medium supplemented with NAA (2.63 μM) (Fig 26-a). Similar results were found with *Fragreria anannassa* where best rooting was obtained on MS medium with NAA (1.0 μM) (Bhatt & Dhar, 2000) and 94% shoots induced roots on a medium containing NAA (2.7 μM) in a monocot Sego Lily (Hou et al, 1997). During the *in vitro* stage improved acclimatization frequency and further growth in the horticultural substrate.

Whereas, lower concentrations than 10 μM of IAA and IBA failed to produce any rooting in the *in vitro* regenerated shoots of *Schefflera arboricola*. In many of the

Fig 25: Multiple shoot induction in *Schefflera arboricola.*

(a) Multiple shoot induction on MS solid basal medium containing BA (8.87 μM).

(b) Extension growth of multiple shoots noted after 21 days of incubation.

(c) Further growth of multiple shoot on MS basal medium.

In figures, the bar = 1 cm


a
b
c

ornamental species auxins IAA and IBA have responded well for the production of the roots from the *in vitro* grown shoots.

Table-7.4: Effect of multiple shoot induction response and their growth on MS medium containing BA and KN individually and in combination from *in vitro* derived nodal explants of *Schefflera arboricola* after 15 days of incubation.

CYTOKININ (μM)		MULTIPLE SHOOT RESPONSE (%)* MEAN ± S.E.	MULTIPLE SHOOTS / PLANT* MEAN ± S.E.	SHOOT LENGTH (cm)* MEAN± S.E.
Basal medium		-	-	-
BA	KN			
1.10	-	18 ± 2	1.2 ± 0.9	0.52 ± 0.09
2.21	-	25 ± 1.5	1.8 ± 0.6	0.55 ± 0.07
4.43	-	27 ± 2.7	3.0 ± 0.8	0.65 ± 0.1
6.65	-	48 ± 1.9	2.1 ± 1.2	1.4 ± 0.1
8.87	-	67 ± 3.2	5.6 ± 1.5	1.0 ± 0.1
-	1.16	50 ± 2.2	4.5 ± 1.7	0.4 ± 0.07
-	2.32	48 ± 1.6	4.1 ± 2.1	0.58 ± 0.09
-	4.64	33 ± 2.2	5.1 ± 0.8	0.6 ± 0.09
-	6.96	47 ± 2.3	3.4 ± 0.7	0.75 ± 0.09
-	9.29	37 ± 2.3	2.8 ± 6.2	0.63 ± 0.1
2.21	1.16	30 ± 3.5	2.6 ± 5.8	0.87 ± 0.4
4.43	1.16	22 ± 2.9	3.0 ± 1.2	0.45 ± 0.07
8.87	1.16	16.3 ± 4.2	2.1 ± 1.2	0.32 ± 0.07

± S.E. – Standard error

*Values are the percentage means of 3 independent experiments.

Table-7.5: Effect of the different Auxins (IAA / NAA / IBA) in MS medium on rhizogenesis of *in vitro* grown shoots of *Schefflera arboricola* after 15 days of incubation

AUXINS (μM)	NUMBER OF ROOTS* MEAN ± S.E.	ROOT LENGTH (cm)* MEAN ± S.E.
Control	-	-
IAA		
1.42	-	-
2.85	-	-
5.70	-	-
11.41	0.5 ± 0.02	0.3 ± 0.07
NAA		
1.34	-	-
2.63	2.5 ± 0.3	1.6 ± 0.2
5.37	1 ± 0	0.85 ± 0.03
10.74	-	-
IBA		
1.23	0.5 ± 0.2	0.35 ± 0.2
2.46	-	-
4.92	-	-
9.84	1 ± 0	0.45 ± 0.1

± S.E. – Standard error
*Values are the percentage means of 3 independent experiments

Acclimatization of plantlets and transfer to the field: The rooted shoots were transferred to the plastic net pots (2 cm diameter) containing sterile mixture of vermiculite and perlite (1:1; v/v) (Fig 26-b) and covered with polythene bag in the culture room for 15 days. Such *in vitro* derived rooted plantlets were gradually exposed to the culture room conditions by making small holes (4 mm) in the polythene bag and gradually increasing their number for acclimatization. After this they were exposed to 2 to 3 hrs outside the culture room. Thus after thorough hardening the plantlets were transplanted to small earthen pots (8 cm diameter) containing a mixture

Fig 26: Induction of root and establishment of in vitro raised plants of *Schefflera arboricola.*

(a) Induction of roots on shoots in MS basal medium supplemented with auxin NAA (2.63 μM) after 15 days of incubation.

(b) Rooted shoots in plastic pot (2 cm diameter) containing vermiculite and perlite (1:1).

(c) Plantlets transferred to the earthen pots (8 cm diameter) before its field transfer.

In figure, the bar = 1 cm


a
b
c

of garden soil and compost (1:1; v/v) and transferred under the shade of *Polyalthia longifolia* (Sonn) Thw. in the Botanical garden (Fig 26-c). There the plants were frequently subjected to mist-irrigation with spraying bottle (1 litre capacity) accompanied by fans for circulating the cool air. Survival of regenerated plants in the field was 60-70 %.

Field study of *in vitro* regenerated plants: Once the *in vitro* regenerated plants were transferred to the field, the physical appearance of the regenerated and conventionally propagated plants in the field was found to be similar. There was no significant difference in the morphology of *in vitro* and *in vivo* raised plants i.e. shape, size and arrangement of leaves on the stem. However, the low survival rate of *in vitro* regenerated plants may be due to the lack of shock adaptation during acclimatization from *in vitro* to *ex vitro* conditions (Smith and Hamil, 1996).

Thus from this protocol almost 150 shoots of *Schefflera arboricola* were produced from single axillary node after a 3 month cycle. These were further rooted and acclimatized before transferring to field conditions.

Flow chart (Fig 27) for the clonal multiplication of *Schefflera arboricola* is included in the following page:

Fig 27: Flowchart for clonal multiplication of *Schefflera arboricola*