Chapter VI

IN VITRO STUDIES WITH *Aglaonema commutatum* Schott.

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Introduction:

Origin and distribution: *Aglaonema* is a genus of ornamental herbs, belonging to Araceae family distributed in the Indo-Malaysian region. There are several species among which five are reported from India.

General description: Petiole succulent; leaves leathery oblong-lanceolate, deep green or variegated, 20 cm or longer. Petiole succulent; waxy-white spathe. Pale green or white flowers; capitate berries yellow to bright red.

Ornamental value: *Aglaonema* species are succulent or shrubby perennials valuable as pot plants. *A. commutatum* Schott, *A. hookerianum*, *A. nocobarium* Hook and *A. pictum.* Kunth and several other species are cultivated for their glossy, variegated leaves and bright red berries (Anonymous, 1972). The production of *Aglaonema* has increased in recent years from less than 1 % of foliage plant production in the 1960s to more than 6 %. *Aglaonema modestum* (Chinese evergreen) and *Aglaonema commutatum* **Treubii** (Ribbon evergreen) were the major cultivars, but many cultivars generated by plant collectors and breeders, have recently become more important (Henny et al, 1991).

Important *Aglaonema* cultivators listed in the Florida Foliage Plant Locator are:

A. commutatum	*A. commutatum* **Emerald Beauty**	
A. costatum	*A. crispum*	*A. modestum*
A. 'Abidjan'	*A. 'B.J.Freeman'*	*A. 'Bangkok'*
A. 'Fransher'	*A. 'Lilian'*	*A. 'Malay Beauty'*
A. 'Manila'	*A. 'Maria'*	*A. 'Parrot Jungle'*
A. 'Romana'	*A. 'San Remo'*	*A. 'Silver Duke'*
A 'Silver King'	*A. 'Silver Queen'*	

Different species of *Aglaonema:*

***Aglaonema commutatum* Schott.:** (Philippines, Sri Lanka) **Silver evergreen** A plant with spotted and greyish, oblong lanceolate leaves (Fig 18-a), pale green or white flowers (Fig 18-b), and capitate berries, is of special merit to horticulturists. It is a durable plant with leathery, oblong-lanceolate leaves, deep green with markings of silver-grey, 20 cm or longer. Waxy-white spathe; berries yellow to red.

***Aglaonema commutatum* elegans: Variegated evergreen,** robust-growing plant with long, lanceolate leaves deep with greenish-grey feather design.

***Aglaonema commutatum:* Tricolor,** relatively broad deep-green leaves with silver feathering, borne on pink petioles; waxy pinkish spathe and white spadix.

Aglaonema costatum: (Malaya) **Spotted evergreen** a short plant with leathery, shiny, cordate-pointed leaves with broad, white center band, slow growing.

***Aglaonema costatum* var Immaculatum:** (Thailand), handsome erect, spear-shaped leaves, 20-30 cm long, glossy deep green and with prominent white midrib.

***Aglaonema modestum*:** (Kwangtung), **Chinese evergreen** with durable leathery, waxy-green leaves, ovate-accuminate and to some extent, pendant, on a slender cane spathe-green, spadix cream.

***Aglaonema modestum*:** 'medio-pictum' (pat as schingii) **Mandalay plant** (Mahaffey,1967), an attractive and sharply distinct variegation, of leaf color - yellow chartreuse or apple green and ivory along center, which abruptly changes to dark green around outer edge of leaf, on variegated petioles. It is an excellent ornamental plant with stocky cane and of compact habit, freely branching, ovate-pointed, leathery glossy foliage, with leaves 12-25 cm long of good keeping qualities.

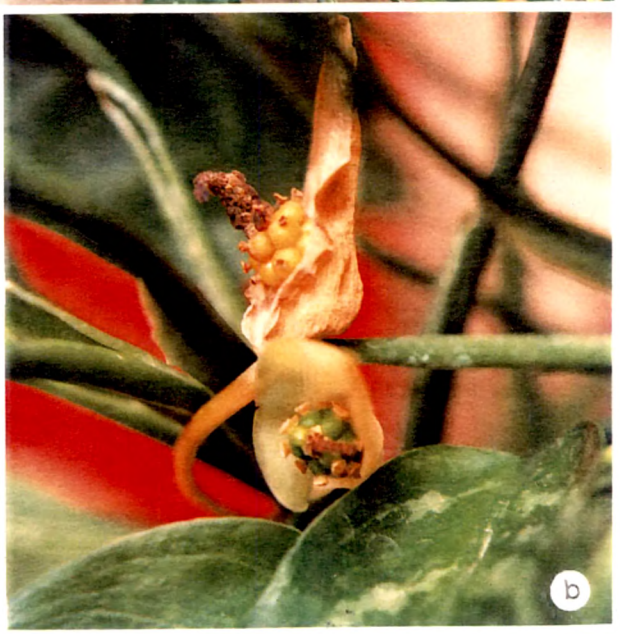
***Aglaonema modestum* var Variegatum:** cultivar from Pennock's Puerto Rico nursery has leathery lanceolate deep green leaves dominated by creamy variegation.

Fig 18: *Aglaonema commutatum* Schott. for micropropagation studies.

(a) Plants growing in the Botanical garden of the M S University of Baroda selected.

(b) Inflorescence of *Aglaonema commutatum* Schott. There is no seed formation in Baroda conditions.


a


b

***Aglaonema pictum*: Tricolor** (*Versicolor*) (Sumatra), deep green, satiny leaves with patches of silver and mixed with yellow-green blotches.

Aglaonema pictum: 'pseudo-bracteatum' **Golden evergreen,** colorful, free growing mutation or hybrid with long, showy leaves deep green variegated with light green and yellow, center largely cream-white; stem white and marbled green; waxy greenish-white spathe and cream spadix.

***Aglaonema pictum* Kunth.:** The Malaysian people eat the leaves as a vegetable and it is found in Burma, Malaysia and Sumatra region (Anonymous, 1935).

Aglaonema rotundum: (Malay peninsula) **Red *Aglaonema,*** compact, slow growing beauty which was found in northern Thailand; with stout stem covered by sheathing short petioles, leaves broad-ovate or rhombic, 10-15 cm or more long, thick-leathery, dark metallic glossy palling; the reverse side glowing wine-red with rosy veins.

Aglaonema siamense: **Silver Queen,** very ornamental and decorative plant, from the same Florida hybrid complex as a **Silver king** with leaves narrower, about 22 cm long and 3 to 5 cm wide; green with grey marbling in feather pattern, petioles more green; freely branching and suckering, for bushy effect, and of excellent keeping qualities

Aglaonema treabii*: Ribbon *Aglaonema, slender plant with narrow, leathery, bluish-green leaves attractively marked with silver-greyish marbles on petioles (Anonymous, 1986).

Medicinal value of *Aglaonema:*

***Aglaonema commutatum* Schott.:** The leaves are used to reduce swellings, they contain α and ß carotenes. Small amounts of carotenoids are present in the floral buds too. The fruits contain the following carotenoids -; Lycopene, Lycoxanthain,

Violaxanthin, 4-keto-3-hydroxylycopene and α - β - γ- and δ- carotenes. The presence of lutein is also reported in the seeds (Anonymous, 1994; Burkil, 1976).

***Aglaonema pictum* Kunth.:** It has medicinal value as it is considered to be anti-thelmentic and as a tonic for bronchitis (Anonymous, 1994).

Cultural requirements: *Aglaonema* grow best in fairly heavy shade of 73 - 90% with the highest shade level required where temperatures may exceed 35° C. Excellent growth can be obtained with liquid or slow-release fertilizer when applied. (Poole et al, 1990). Different micronutrients are required for *Aglaonema*, especially copper, since deficiency of this element is common.

Potting media and soil temperature: Potting media utilized must have excellent aeration; *Aglaonema* does not grow well in heavy, wet mixtures, although ample soil moisture is necessary. Good growth occurs when soil temperatures are 21° C-29° C, with similar air temperatures. Limited growth will occur at 18° C soil temperature, but any lower temperature results in poor or no growth (Henny et al., 1991).

Propagation: *Aglaonemas* are easily raised from the terminal or axillary node cuttings of the stem and by divisions of the basal shoot (Poole, 1983). *Aglaonemia pictim* Kunth is also medicinally useful. The leaves are eaten as a vegetable in Malaysia to be anti-thelmintic and tonic (Burkil, 1976; Anonymous 1994).

Physiological problems: Some of the common physiological problems occurring in *Aglaonema* are:

Chilling Injury:

Symptoms and Causes: Mainly mid to older (lower) leaves develop grey splotches and become chlorotic; lower leaves may collapse after 3 to 7 days if damage is severe. **Silver Queens** is especially sensitive to cold.

Control: Keep **Silver Queen**, not less than 13° C to prevent damage, and most other cultivars at 7°C to 10° C. The damage is permanent, but damaged plants will

continue to grow unless terminals are affected by extreme cold. (Fooshee et al, 1987)

Copper Deficiency:

Symptoms and Causes: Terminal leaves become chlorotic and sometimes even dwarfed and deformed, with serrated edges. Older leaves become light green than normal. Certain varieties like **Fransher** are especially susceptible to copper deficiency.

Control: Application of copper sulphate to soil surface, or copper sprays to foliage are effective. Copper is included in the potting medium or a periodic micronutrient application of copper is used. Soil temperatures of 18°C or below will contribute to copper from cold soils. Thus, soil temperature should be raised or foliar copper should be applied during such periods (Poole et al, 1979)

Excess Light And Temperature:

Symptoms and Causes: Leaves assume more or less vertical or low angle position instead of the normal 45 to 90° angle from the stem, leaf colour will also be light or display a washed-out appearance, and in extreme cases, leaf tips will be whitish or pale.

Control: By providing recommended light and temperature levels, leaves will reassume their normal position. Severely bleached leaves may not fully recover (Conover, 1990).

Bent-Tip:

Symptoms and Causes: The terminal leaf spike will have a fishhook appearance, and some older leaves will also have a hook at the terminal. The new leaf tip appears to be obstructed and caught by the succeeding leaf, resulting in the fishhook appearance.

Control: Not known, although excessive light and water stress have been observed to increase severity in susceptible cultivars.

Bacterial problems:

Bacterial blights and stem rots (*Erwinia carotovora, E.chrysanthemi*):

Symptoms and Causes: Bacterial blight is typified by watery leaf spots with centres that frequently disintegrate. Bacterial stem rots caused by *Erwinia spp* are generally noticed first, following sticking of cuttings. At this time, the cut end of the stem becomes mushy and foul smelling and the rooting process is delayed if not all together halted. The cuttings usually become yellow quickly.

Control: Control of bacterial leaf spots or blight can be accomplished through use of clean propagation material and watering system that either does not wet the foliage or allows it to dry rapidly. Both antibiotic and copper compounds may aid in control if applied weekly during the summer months when the disease is most severe. Bacterial stem rot is usually not possible to control once started. Use of clean propagation material is the only successful method of cultural control although some growers have reported rouging infected plants and recutting variable ones prior to dipping in agri-step as moderately successful control methods.

Xanthomonas Leaf Spot (*Xanthomonas ampestris*):

Symptoms and Causes: Reddish-brown areas on edges of leaf with bright yellow margins are the most common symptoms. Under wet and warm conditions, bacteria also spread into leaf centres and lesions expand until they reach a leaf vein. Sometimes lesions are also small, water-soaked, specks, which enlarge into irregularly shaped areas.

Control: Minimize foliage wetting and use of pathogen free stock materials. Foliar applications of copper or antibiotic compounds on a weekly basis provide adequate control under same conditions (Henny, 1991).

Fungal problems:

Fusarium Stem Rot (*Fusarium Spp.*):

Symptoms and Causes: *Fusarium* stem rot typically appears as soft, mushy rot at the base of a cutting or rooted plant. The rotten area frequently has a purplish to reddish margin. *Fusarium* sometimes forms tiny bright red, globular structure (fruiting bodies) at the stem base of severely infected plants.

Control: Stem or cutting rot is a problem, treatment of the cuttings with a dip or a post-sticking drench should diminish losses. Remove infected plants from stock areas as soon as they are detected. Since *Fusarium* stem rot appears similar to *Erwinia* blight, accurate disease diagnosis is very important prior to applications of pesticides (Simone et al, 1989).

Myrothecium Leaf Spot (*Myrothecium roridum*):

Symptoms and Causes: *Myrothecium* leaf spot is one of the easiest foliage diseases to diagnose. Leaf spots are generally found at wounds, although it is common to find no obvious wound and very large (up to 1 inch) leaf spots. The spots are tan to brown and may have a bright yellow border. Examination of the lower leaf surface shows the black and white fruiting bodies of the pathogen in concentric rings near the outer edge of the spot.

Control: Control can be achieved if plant foliage is maintained dry and wounding is eliminated.

Root Rot (*Phytium spp.*):

Symptoms and Causes: Root rot is typified by wilting of plants and yellowing of lower leaves. The roots themselves are brown to black, reduced in mass and mushy. The outer portion of infected roots can easily be pulled away from the inner core.

Control: Use of pathogen free potting medium and pots and growing plants on raised benches, can eliminate much of this problem. Since, many times other pathogens are also involved, accurate diagnosis of the cause must be made prior to choice of fungicides.

Insect and related problems:

Agloanema does not appear to be sincerely affected by insect, mite or related pests, with the possible exception of periodic infestations of caterpillars (larvae) of *Lepidopterous* insects, mealybugs as well as *Aglaonema* and *latania* scales.

Mealybugs:

Symptoms and Causes: Mealybugs appear as white, cottony masses in leaf axils, on the lower surfaces of leaves and on the roots. Honeydew and sooty mold are often present and infested plants become stunted, with severe infestations, finally plant parts begin to die.

Control: Systemic materials are preferred.

Scales:

Symptoms and Causes: Infected plants become weakened or stunted and begin to die. Scale can be found feeding on leaves, petioles or stems. Their shapes, sizes and colours are variable and many are hard to distinguish from the plant material on which they are feeding.

Control: Systemic materials are preferred.

Caterpillars (worms):

Symptoms and Causes: Infestations are easy to detect because worms, their excrement and the damage they cause, are usually quite visible to the unaided eye. Damage appears as holes in the centre or along the edges of leaves. Old damage can be distinguished from new by the calloused appearance of the older areas.

Control: Several acceptable products for worm control are available as reported by Short and Borne (1989).

The widespread use of micropropagation has many advantages. Adult plant material, which often cannot be cloned *in vivo*, sometimes can be rejuvenated *in vitro* and subsequently cloned. Micropropagation or *in vitro* methods of regeneration have been used extensively for the propagation of horticultural crops and plantation crops. Many *in vitro* techniques are useful to produce disease free planting material.

Materials and methods:

Plant Material: The young shoots were collected from the healthy plants of *Aglaonema commutatum* Schott. growing in pots in the Botanical Garden of the M. S. University, Baroda. After removing leaves with sheathing leaf bases, their cut ends were immediately dipped in water. The shoots with 6-8 nodes were cut into single nodal explants and apical buds.

Sterilization of explants: The explants were washed with running tap water for about 15 minutes to remove dust particles and then treated with mild detergent Teepol (1%; v/v) for 15 min and again kept under tap water for 20 min. This was followed by the pre-treatment of explants with Bavistin (1%) and Rifampcin (0.75 %) for about 3 – 4 hr on shaker (100 rpm) before inoculation to reduce contamination. After this the explants were washed 3 times with distilled water and further treated with sterilant $HgCl_2$ (0.1 %) for 6 minutes in the Laminar Flow Hood and subsequently followed by 3 to 4 washes with sterile distilled water. For multiple shoot induction the sprouted explants were given different types of incisions in the sprouted bud. The incisions made with sterile blade were of two types:

- ⇨ Single vertical incision in the plane of bud (E1) (↕)
- ⇨ Two vertical incision intersecting each other in the bud (E2) (✣)

Both the nodal and apical explants were cultured on MS medium (Murashige and Skoog, 1962). The pH was adjusted to 5.8.

Initiation of cultures: For bud-break cytokinins BA and KN on MS basal medium was tried. Multiple shoot induction was tried on MS medium supplemented with BA alone or with various concentrations of auxins – IAA / NAA. Shoot tip culture and different type of incisions (E1 and E2) in the bud were the methods applied for multiple shoot induction. Rooting media was not used as *in vitro* roots were induced from the axils in the multiplication medium and *ex vitro* roots developed in

the pots during acclimatization procedure. Acclimatization and hardening of regenerated plants was achieved similarly as described in chapters 2, 3 and 4.

Results and discussions:

Axillary bud sprouting: Contamination free cultures were established on MS solid basal medium and after a week were transferred to the Bud initiaton medium.The axillary bud explants collected during September to January showed sprouting, on 6^{th} - 7^{th} day of inoculation on MS medium supplemented with BA (Fig 19-a). Almost 72% of the nodal explants sprouted on an average on MS medium with BA (0.88 μM) compared to only 30 % sprouting on MS medium supplemented with KN (0.92 μM) (Table-6.1). The roots were also produced in the bud initiation medium (Fig 19-b).

Increase in the concentration of both the cytokinins i.e. BA and KN showed decrease in rate of bud sprouting from axillary bud explants. The shoots produced in the medium supplemented with BA were quite sturdy and healthy than those produced in the KN supplemented medium. From the results, thus it is clear that BA is a better cytokinin than KN for bud initiation. The synthetic cytokinin BA is routinely added to tissue culture media to stimulate shoot proliferation and is most commonly used cytokinin commercially (Blakesley and Constantine, 1992). BA has also been used for the axillary bud initiation for most of the ornamental species as in the case of *Bougainvillea spectabilis* and *Syngonium podophyllum* (Misra et al, 1997), *Mussaenda luteola* (Jasrai et al, 1999) blue Vanda (Seeni and Latha, 2000) and Rose (Hasegawa, 1979).

The statistical analysis by paired t test revealed the results to be highly significant for bud-break response induced on two different cytokinins BA / KN. Thus there is a significant difference between the bud-break response among both the cytokinins used BA and KN.

Table-6.1: Effect of cytokinins (BA / KN) on bud sprouting response of nodal explants of *Aglaonema commutation* Schott. on MS medium supplemented with BA (0.88 μM) after 7 days of incubation.

CYTOKININS (μM)	BUD SPROUTING* MEAN ± S.E.
BA	
0.44	16.6 ± 7.8
0.88	72.1 ± 3.5
2.21	45.2 ± 7.0
4.23	49.9 ± 3.3
8.87	29.9 ± 2.7
KN	
0.46	29.6 ± 2.7
0.92	25.7 ± 3
2.32	16.7 ± 1.6
4.64	11.6 ± 3.5
9.29	13.2 ± 1.4

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

	LEVENE'S TEST FOR EQUALITY OF VARIANCES		T-TEST FOR EQUALITY OF MEANS		
	F	Sig	T	df	Sig. (2 tailed)
Equal variances assumed	9.777	.002	5.455	133	.000**
Equal variances not assumed			6.045	115.226	.000**

*The data was subjected to paired t test at $p \leq 0.05$ significance
**Significant

Multiplication of shoots: Bud sprouts of *Aglaonema* on MS basal medium supplemented with BA (0.88 μM) were used for further multiplication. The protruding buds were subjected to two types of incisions E_1 - single vertical incision in the plane

of bud (↕) and E_2 - two vertical incisions intersecting each other (✣). The explants with incisions when transferred to MS basal medium supplemented with BA (2.32 μM) showed no response for the induction of multiple shoots, both the type of incisions failed to induce any multiple shoot formation. However, those buds with E_2 type of incisions showed some swelling at the bud surface without any further development.

The explants with incisions were then transferred to MS media supplemented with various concentrations of cytokinin - BA, auxins - NAA / IAA. However, both auxins IAA / NAA with BA could not induce high multiple shoots. Although among both the auxins tried, it was observed that IAA in combination with BA was found suitable compared to the medium supplemented with NAA and BA, for the induction of multiple shoots and their growth (Table-6.2). Maximum initiation of 3 multiple shoots were noted on MS medium containing IAA (0.571 μM) with BA (2.21 μM) (Fig 19-c). Maintaining the same levels of auxin and reducing the levels of BA could not induce multiple shoots. In other combinations of IAA with BA only swelling was observed at the bud surfaces. There was no significant growth of these multiple shoots, when transferred to fresh cultures. Thus IAA and BA interaction could only help in the initial growth but not during the later stages of their growth. Similar results were observed in *Gardenia jasminoides* (George et al, 1993) for the growth of axillary buds. This may be due to the fact that morphogenetic expressions in a particular direction is manifested as a result of the cumulative effect of the subtle interactions and balances among the growth regulator substances and nutrient constituents mediated through various physical factors (Halperin, 1969). The elongation of the multiple shoots was achieved on the basal medium (Fig 19-d), which were further rooted and acclimatized.

Meristum culture: The meristem culture was also tried for multiplication. The meristems were isolated from the shoot apices (Fig 20-a) were inoculated on MS medium containing BA (2.21μM, 5.37μM and 10.7μM). A little swelling was observed at the meristem base but it failed to give rise to any shoot formation (Fig 20-b).

Fig 19: Establishment, growth of axillary buds and multiple shoot induction in *Aglaonema commutatum* Schott.

(a) Axillary bud initiation on MS medium supplemented with BA (0.88 μM) after an incubation for a week.

(b) Root initiation alongwith bud growth on the same medium as in (a) after 7 days of incubation.

(c) Multiple shoot induction by incision (E_2) in the sprouted bud on MS solid medium containing IAA (0.571 μM) and BA (2.21 μM). (Note: Root initiation from the axil in the same medium).

(d) Elongation growth of multiple shoots on MS basal solid medium.

In figures, the bar = 1 cm

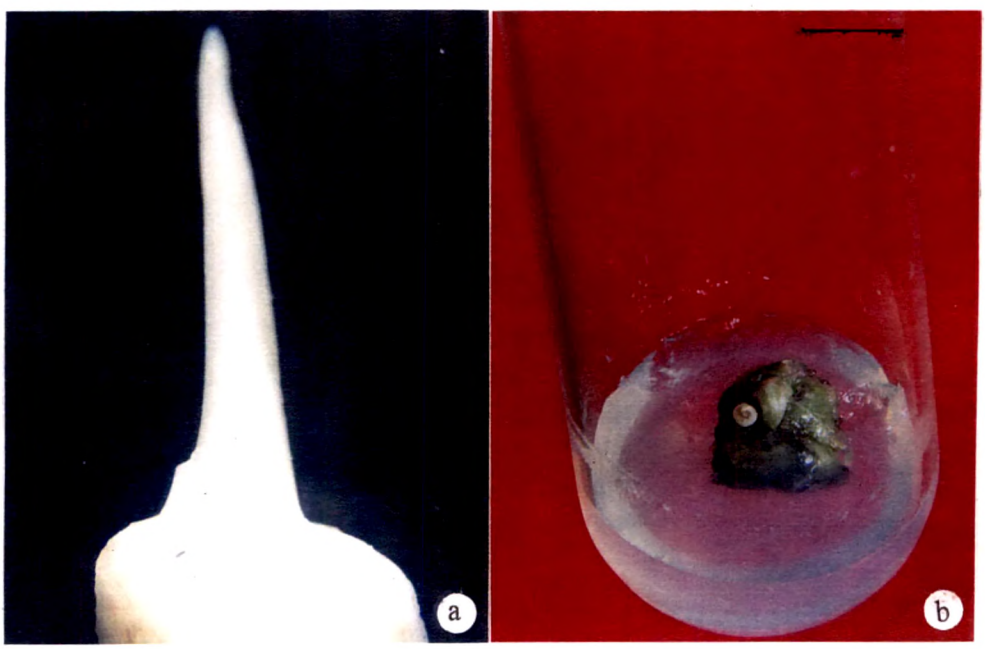

a
b
c
d

Fig 20: Apical bud culture in *Aglaonema commutatum* Schott.

(a) Apical meristem isolated (4 to 5 mm).

(b) Apical meristem incubated on MS medium supplemented with BA (5.37 μM); failed to give response.

In figures, the bar = 1 cm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b

Root induction: Rooting was achieved in the same medium, which induced shoot formation (Table-5.2). As axillary explants always have the presence of root initials. Similar type of rooting was achieved in *Syngonium* (Misra et al, 1997). Amongst both the auxins tried in combination with BA in MS basal medium for rooting, all the concentrations of BA with NAA on MS basal medium induced roots on shoots. However, on an average maximum 2 roots were produced with 0.91 cm root length on MS medium containing auxin-NAA (0.52 μM) and BA (1.10 μM). Whereas, most of the concentrations of BA with IAA could not produce any roots. The *ex vitro* rooting was also achieved during hardening of shoots when transferred to the plastic pots containing a mixture of vermiculite, perlite and sand (2:1:1) as similar to the other *monocot Amaryllis* where *ex vitro* rooting was achieved when transferred to potted soil (Prasad and Chaturvedi, 1993).

Table-6.2: Effect on multiple shoot and root induction from buds cultured on different growth media in *Agalonema commutatum* Schott. Data recorded after 15 days of incubation.

GROWTH REGULATORS (μM)		NUMBER OF MULTIPLE SHOOTS* MEAN ± S.E.	SHOOT LENGTH (cm)* MEAN ± S.E.	NUMBER OF ROOTS/ SHOOT* MEAN ± S.E.	ROOT LENGTH (cm)* MEAN ± S.E.
NAA	BA				
0.53	0.88	-	-	1.27±0.01	1.06±0.02
	1.10	-	-	1.3 ± 0.07	0.91±0.12
	2.21	-	-	0.5±0.37	0.7±0.49
IAA					
0.57	0.88	1±0	0.53±0.07	0.5±0.37	0.25±0.17
	1.10	0.6±0.57	0.56±0.24	-	-
	2.21	2.6±0.57	0.65±0.1	-	-

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

Acclimatization of plantlets: The healthy shoots of 4 to 6 cm were transferred to the plastic pots (2 cm diameter) containing vermiculite, perlite and sand

Fig 21: Acclimatization of *in vitro* raised plantlets of *Aglaonema commutatum* Schott.

(a) Plantlets in the plastic pots in a tray covered by polythene sheet.

(b) Plantlet in the earthen pot (8 cm diameter).

(c) Plants transferred to field after 1 month.


a
b
c

(2:1:1; v/v), kept in a tray with three layers of filter paper beneath and covered with polythene sheet (Fig 21-a) in which 5 to 6 holes (4 mm) size were made so as to expose them to the culture room conditions. Slowly and gradually the number of these holes were increased. The plants were irrigated every alternate day with distilled water. After keeping the plantlets for 15 days in the tray they were transferred to the earthen pots (8 cm diameter) containing garden soil and compost (1:1;v/v) (Fig 21-b) for further growth and then subsequently transferred to the field after a week (Fig 21-c).

Field study of regenerated plants: After transferring the plantlets to the field, their regular growth was continuously observed. Two months after their field transfer the plants showed vigorous growth with emergence of new leaves. The morphological characters of regenerated plants such as leaf shape; colour and its variegation were found to be very similar with the parental plants indicating that *in vitro* regenerated plants were clonally propagated.

Thus from this micropropagation protocol about 189 plantlets were produced from single node explant in 8–9 weeks.

The flow chart (Fig 22) for the clonal multiplication of Aglaonema commutatum Schott. is included in the following page.

Fig 22: Flow chart for the clonal multiplication of *Aglaonema commutatum* Schott.