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Standardization of protocol for explant preparation and plant regeneration from apical bud and nodal explants of *Anthocephalus cadamba*

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Abstract

Experiment was carried out for disinfection of apical bud and nodal explants of mature tree of *Anthocephalus cadamba*. Two commonly used disinfectants such as NaOCl (1%) and $HgCl_2$ (0.1%) was tested for different duration ranging from 1 – 10 min and disinfection of apical bud and nodal explants with $HgCl_2$ for three min was more effective due to higher recovery of explants for initiation of aseptic cultures. Though NaOCl was effective with increase in duration of treatment, loss of explants due to browning followed by leaching of chlorophyll was observed in actively grown apical buds and nodal segments. The present experiment revealed that $HgCl_2$ was more potent for effective disinfection of apical bud and nodal explants. Experiments conducted with six different antibiotics such as streptocycline, erythromycin, norfloxacin, chloromphenicol, oxytetracycline and rifampicin for control of bacterial contamination revealed promising results. Incidence of bacterial contamination was varying depending upon the type of antibiotics used. All the six antibiotics were found to be effective at their higher concentration above 75 mg/l. Of the six antibiotics tested, streptocycline, erythromycin, chloromphenicol and oxytetracycline were found more effective than norfloxacin and rifampicin. Shoots cultured on MS medium supplemented with different concentrations of antibiotics along with BAP (1 mg/l) had produced healthy shoots without phytotoxic effects and those shoots were converted into complete plantlets by treating the basal end of the microshoots under IBA (5000 ppm) followed by planting of shoots under the controlled condition.

Key words: Plant regeneration, microbial contamination, micropropagation, *ex vitro* rooting.

Introduction

Anthocephalus cadamba Miq (Rubiaceae) is an important fast growing evergreen trees in tropical and subtropical regions in many countries such as Australia, China, India, Indonesia, Phillipines, Singapore, South Africa, Taiwan and Costa Rica. According to Hindu Mythology, this tree is considered as most popular sacred in India (Santapan and Henry, 1973). *A. cadamba* is reported to be a suitable tree for development of agroforestry since this tree sheds large quantities of leaf which are easily decomposed. Leaf litters of this tree contain high organic content and thus improve physical and chemical properties of soil. Dried bark of this holy tree forms an important component in folk medicines for anaemia, uterine complaint besides its several other medicinal properties such as astringent, mucolytic, analgesic, anti-inflammatory, febrifuge and antiseptic (Patel and Kumar, 2008). Mature trees of *A. cadamba* is used in preparation of matchstick boxes, tea boxes, bobbins, veneer, playwood, crates and furniture (Chudnoff , 1984; Zabala *and Manarapaac*, 1968). Thus, the tree is described as gem of tree, wonder tree and miracle tree in Philippines (Lopez, 1966). Depletion of natural population of *A. cadamba* due to its poor seed germination, lack of seed viability and poor efficacy of rooting in conventional method of propagation are the serious concern for conservation of this precious tree (Bose and Chaudhary, 1991).

Failure in propagation of *A. cadamba* by conventional methods by seed and rooted cuttings demands micropropagation techniques for large-scale production of this tree. Preliminary report on regeneration of plantlets by

in vitro culture through somatic embryogenesis was not promising due to lack of reproducibility (Apurva and Thakur, 2009). Further, there was no serious attempt to develop micropropagation protocol for large scale production of *A. cadamba* using apical bud and nodal explants. Regeneration of plantlets using apical bud and nodal explants is one of the safest methods for cloning large number of plants since this method offer true-to-type of plants without genetic variation. *A. cadamba* is a perennial tree and difficult to propagate by tissue culture since the explants sourced from mature tree often encountered with high incidence of microbial contamination. Efficient method of explant preparation is one of the important steps to overcome the problem of microbial contamination. In the present study, two commonly used surface disinfectants such as NaOCl and $HgCl_2$ were tested for their efficacy. In addition few commonly used broad spectrum antibiotics were tested to control the problem of bacterial contamination. Methods were also developed to produce plantlets by *ex vitro* rooting and hardening. The results on this study and its application in conservation of *A. cadamba* are discussed.

Materials and Methods

Source of plant material

About 500 year old well maintained huge tree of *A. cadamba* in Sivasailam Hindu Temple, Alwarkurichi, close to the Western Ghats in southern part of India in Tirunelveli District, Tamilnadu become the mother plant for collection of explants throughout the present investigation (Fig1a). Orthotropic shoots from the mother plant either with active or dormant phase of vegetative growth in different seasons were collected and used for various experiments for developing the protocol for explant preparation and regeneration of shoots from apical bud and nodal segments.

Medium and culture conditions

The basic culture medium used for the present study includes Murashige and Skoog (1962) medium supplemented with sucrose (3%) and various growth regulators depending upon the experimental design. The pH of the medium was adjusted to 5.8 before gelling with 0.8% agar (Hi-media, India). All the chemicals, including growth regulators used in the present study were of analytical grade (British Drug House, Sigma, Merck, and Hi-media). Molten medium was dispensed into 200-ml screw-capped glass jars or 150-ml Erlenmeyer flasks or test tubes (Borosil, India) depending upon the requirements. The culture vials containing the media were autoclaved at 104 kPa and 121°C for 20 min. The processed explants were implanted vertically on the culture medium. All the cultures were maintained at 25±2°C and grown under 16 hr photoperiod with the light intensity of 40 µmol m^{-2} s^{-1} provided by cool white fluorescent tubes (Philips, India). The number of explants cultured in each treatment varied from 50 – 150 depending upon the experiments.

Efficacy of NaOCl and $HgCl_2$ on apical buds

Two commonly used surface disinfectants, namely NaOCl (1%) and $HgCl_2$ (0.1%) were tested for their efficacy in elimination of microbial contamination. The processed apical buds of *A. cadamba* measuring about 1 cm length were surface sterilized with above disinfectants for different durations (1, 2. 3. 5, 7 and 10 min) and were thoroughly rinsed with sterile distilled water for 5 - 6 times under aseptic condition. The basal end of the apical buds were slightly trimmed and implanted vertically on MS medium supplemented with BAP (1 mg/l). Explants cultured without disinfection formed the control. In each treatment, a total of 180 explants were cultured with three replication, each with 60 explants. After 60 days of culture, data on the loss of explants due to microbial contamination and browning, including recovery was recorded and analysed.

Efficacy of NaOCl and $HgCl_2$ on disinfection of nodal segments

A similar experiment as described above was carried out with nodal explants of *A. cadamba*. After disinfection with NaOCl and $HgCl_2$, both the ends of the nodal explants were trimmed under aseptic condition and cultured on the medium as described in the above experiment. Data on microbial contamination was collected after 60 days of culture as similar to the previous experiment and subjected to analysis for optimizing the duration of treatments.

Effect of antibiotics on bacterial contamination

In order to test the efficacy of certain selected antibiotics on bacterial contamination during *in vitro* culture of apical buds of *A. cadamba,* six different commercially used antibiotics such as

streptocycline, erythromycine, norfloxacin, chloramphenicol, oxytetracycline and rifampicin were incorporated into the medium at various concentrations (0, 25, 50, 75, 100 mg/l). These antibiotics were filter sterilized before the use in order to ensure their efficacy. MS medium supplemented with BAP (1 mg/l) become the basic and common medium to all the treatments. In each concentration of antibiotic, 50 explants were cultured. Cultures were incubated under diffuse natural light for 60 days and observation on the incidence of bacterial contamination was carried out and analyzed.

Regeneration of micro shoots and *ex vitro* rooting

Considerable number of microshoots consisting of well developed shoot system with 2 – pairs of leaves was taken from the culture vessels and agar medium adhering on the stem of microshoots were gently removed under running tap water. The basal end of the shoots was trimmed in order to expose the fresh layer of tissues for facilitating the absorption of auxin. Basal end of the shoots were briefly dipped for 5 min in sterile IBA solution (3000 ppm) and implanted on sterile substrate containing soil, sand and vermicompost (6:2:1) and maintained for 45 days. The implanted microshoots were covered with polythene bags and frequently watered to maintain high humidity under natural shade. Shoots that were remained fresh with active shoot growth were removed after 60 days of and observation was carried out to calculate the percentage of rooting.

Statistical analysis

Majority of the experiments were analyzed with three replications. However, the number of explants for the treatment in various experiments was variable due to differences in final recovery of explants. The effect of the different treatments on various parameters was quantified and the level of significance was determinated by analysis of variance (ANOVA) using SPSS version 11.0 and level of differences between the treatments were assessed by Duncan's New multiple range Test (DMRT) at $P \leq 0.05$.

Results and Discussion

Effect of NaOCl and $HgCl_2$ on disinfection of apical buds

Effect of different concentrations of NaOCl and $HgCl_2$ on disinfection of apical bud explants of *A. cadamba* was summarised in table 1 and 2 respectively. In control, all the explants were lost within a week of culture either due to fungul or bacterial contamination. However, explants disinfected with NaOCl (1%) and $HgCl_2$ (0.1%) for different durations showed varying levels of recovery depending upon the duration of treatment. Explants treated with NaOCl and $HgCl_2$ for short durations (1 – 2 min) recorded high incidence of microbial contamination and further increase in duration improved the recovery of explants significantly. Browning of apical bud explants was increased with increase in duration of treatment. In this experiment, disinfection of apical bud explants with NaOCl (1%) recorded the highest recovery with responding explants. However, disinfection of apical bud explants with $HgCl_2$ (0.1%) for 1 – 3 min was more effective than NaOCl (1%). Leaching of chlorophyll from young shoots and leaves was noticed when apical shoots were subjected to disinfection with NaOCl (Fig. 1b). Exposure of apical bud explants to $HgCl_2$ for longer duration (3 – 7 min) did not reveal any phytotoxic effect as evidenced by improved bud break and shoot regeneration.

Effect of NaOCl and $HgCl_2$ on disinfection of nodal explants

Results on the efficacy of NaOCl and $HgCl_2$ on disinfection of nodal explants of *A. cadamba* was summarized in table 3 and 4 respectively. The loss of explants due to fungal contamination was higher than bacterial contamination when nodal explants were disinfected with NaOCl (1%). On the other hand, fungal contamination was reduced significantly in all the treatments when nodal explants were treated with $HgCl_2$. In general, the overall recovery of explants was improved when nodal explants was disinfected with $HgCl_2$ than NaOCl. There was no difference between the treatments of nodal explants with NaOCl and $HgCl_2$ with regard to browning of explants. In general apical bud explants were found to respond well for bud break and shoot regeneration irrespective of the type of disinfectants used. The above experiment clearly revealed that apical buds were more suitable for the initiation of cultures due to higher recovery with better responses over nodal explants. In the present study, bud break and shoot regeneration could be obtained from node and apical bud explants (Fig1.c & d).

Table- 1: Effect of different duration of surface sterilization with NaOCl (1%) on microbial contamination in cultured apical bud explants of *A. cadamba*. Explants were cultured on MS medium supplemented BAP (1 mg/l). Duration of culture 60 days.

Treatments (min)	No. of explants with microbial contamination			Browning	Recovery	Response (%)
	Bacteria	Fungus	Bacteria/ Fungus			
0.0	23.4^{a}	20.3^{e}	9.23^{a}	7.22^{c}	0.00^{f}	0.00^{e}
1.0	8.23^{c}	27.1^{a}	5.33^{f}	3.05^{e}	16.3^{c}	26.6^{c}
2.0	12.1^{b}	27.2^{b}	8.24^{b}	4.33^{d}	9.09^{e}	15.1^{d}
3.0	6.11^{f}	26.1^{c}	6.12^{e}	2.13^{f}	20.3^{a}	33.3^{a}
7.0	7.09^{d}	21.2^{d}	7.08^{c}	9.42^{b}	16.1^{d}	26.6^{c}
10	6.32^{e}	7.09^{f}	6.33^{d}	23.3^{a}	17.3^{b}	28.5^{b}

Response of explants was calculated out of recovered explants.
Data represents the mean values of three replications, each consist of 60 explants. Mean values within each column followed by the same letter in superscript are not significantly different (P≤ 0.05: Duncan's New Multiple Range Test).

Table 2: Effect of different duration of surface sterilization with $HgCl_2$ (0.1%) on microbial contamination in cultured apical bud explants on MS medium supplemented BAP (1 mg/l). Duration of culture 60 days.

Treatments (Min)	No. of explants with microbial contamination			Browning	Recovery	Response (%)
	Bacteria	Fungus	Bacteria/ Fungus			
0.0	26.7^{a}	10.9^{a}	13.1^{a}	9.33^{c}	0.00^{f}	0.00^{f}
1.0	2.84^{e}	2.22^{d}	3.07^{f}	3.10^{e}	49.0^{a}	81.6^{a}
3.0	5.25^{b}	4.22^{b}	4.22^{c}	3.10^{e}	44.1^{b}	73.3^{b}
5.0	3.87^{d}	1.93^{e}	3.33^{e}	8.43^{d}	42.7^{c}	70.0^{c}
7.0	4.74^{c}	1.64^{f}	6.14^{b}	15.1^{b}	32.4^{d}	53.3^{d}
10	4.65^{c}	3.13^{c}	4.15^{d}	23.3^{a}	25.7^{e}	45.1^{e}

Response of explants was calculated out of recovered explants.

Data represents the mean values of three replications, each consist of 60 explants. Mean values within each column followed by the same letter in superscript are not significantly different (P≤ 0.05: Duncan's New Multiple Range Test).

Table - 3: Effect of different duration of surface sterilization with NaOCl (1%) on microbial contamination in cultured nodal explants on MS medium supplemented BAP (1 mg/l). Duration of culture 60 days.

Treatments (min)	No. of explants with microbial contamination			Browning	Recovery	Response (%)
	Bacteria	Fungus	Bacteria/ Fungus			
0.0	22.1^{a}	20.1^{e}	10.2^{a}	8.22^{c}	0.00^{f}	0.00^{f}
1.0	9.22^{d}	28.1^{a}	6.32^{f}	4.03^{e}	13.3^{b}	22.1^{b}
2.0	12.1^{b}	27.2^{b}	8.22^{b}	5.32^{d}	8.02^{e}	13.0^{e}
3.0	6.13^{f}	26.1^{c}	7.13^{e}	3.13^{f}	18.2^{a}	30.0^{a}
7.0	8.03^{e}	22.2^{d}	8.02^{c}	10.4^{b}	12.1^{c}	20.0^{c}
10	9.32^{c}	9.02^{f}	7.32^{d}	25.3^{a}	10.0^{d}	16.6^{d}

Response of explants was calculated out of recovered explants.
Data represents the mean values of three replications, each consist of 60 explants. Mean values within each column followed by the same letter in superscript are not significantly different (P≤ 0.05: Duncan's New Multiple Range Test).

Table 4: Effect of different duration of surface sterilization with $HgCl_2$ (0.1%) on microbial contamination in cultured nodal explants on MS medium supplemented BAP (1 mg/l). Duration of culture 60 days

Treatments (Min)	No. of explants with microbial contamination			Browning	Recovery	Response (%)
	Bacteria	Fungus	Bacteria/ Fungus			
0.0	29.7[a]	10.9[a]	12.1[a]	7.32[d]	0.00[f]	0.00[f]
1.0	6.82[d]	4.23[f]	5.03[e]	7.02[e]	36.5[a]	61.6[a]
3.0	9.23[c]	8.23[b]	6.28[c]	5.03[f]	32.0[b]	53.3[b]
5.0	10.8[b]	4.93[e]	6.33[c]	9.43[c]	28.7[c]	47.8[c]
7.0	6.72[e]	5.63[d]	8.11[b]	18.1[b]	22.4[d]	37.3[d]
10	6.62[f]	6.02[c]	6.14[d]	25.3[a]	16.7[e]	27.8[e]

Response of explants was calculated out of recovered explants.

Data represents the mean values of three replications, each consist of 60 explants. Mean values within each column followed by the same letter in superscript are not significantly different (P≤ 0.05: Duncan's New Multiple Range Test)

Table -5: Effect of different antibiotics on microbial contamination in cultured apical bud explants of *A. cadamba*. Explants were cultured on MS medium supplemented BAP (1mg/l) and with different concentrations of antibiotics. Duration of culture 60 days.

Antibiotics mg/l						Contamination					
Str	Ery	Nor	Chl	Oxy	Rif	Bacteria	Fungal	Bacteria/Fungal	Browning	Recovery	Response (%)
-	-	-	-	-	-	29.4[a]	6.40[ef]	3.40[h]	4.40[g]	8.40[j]	16.4[m]
25	-	-	-	-	-	14.4[bc]	6.40[ef]	7.40[de]	9.40[de]	14.4[h]	28.4[j]
50	-	-	-	-	-	12.4[de]	9.40[bc]	5.40[fg]	9.40[de]	15.4[gh]	30.4[i]
75	-	-	-	-	-	7.40[i]	8.40[cd]	6.40[ef]	10.4[d]	19.4[ef]	38.4[f]
100	-	-	-	-	-	11.4[ef]	7.40[de]	4.40[gh]	12.4[c]	16.4[g]	32.4[h]
-	25	-	-	-	-	13.4[cd]	5.40[fg]	5.40[fg]	8.40[ef]	19.4[ef]	38.4[f]
-	50	-	-	-	-	10.4[fg]	7.40[de]	4.40[gh]	7.40[f]	22.4[bc]	44.4[c]
-	75	-	-	-	-	5.40[j]	6.40[ef]	5.40[fg]	7.40[f]	27.4[a]	54.4[a]
-	100	-	-	-	-	9.40[gh]	5.40[fg]	3.40[h]	10.4[d]	23.4[b]	46.4[b]
-	-	25	-	-	-	14.4[bc]	7.40[de]	9.40[bc]	12.4[c]	8.40[j]	16.4[m]
-	-	50	-	-	-	15.4[b]	11.4[a]	7.40[de]	7.40[f]	10.4[i]	20.4[k]
-	-	75	-	-	-	10.4[fg]	8.40[cd]	5.40[fg]	8.40[ef]	19.4[ef]	38.4[f]
-	-	100	-	-	-	12.4[de]	8.40[cd]	9.40[bc]	7.40[f]	14.4[h]	28.4[j]
-	-	-	25	-	-	15.4[b]	9.40[bc]	10.4[b]	7.40[f]	9.40[ij]	18.4[l]
-	-	-	50	-	-	13.4[cd]	7.40[de]	7.40[de]	8.40[ef]	15.4[gh]	30.4[i]
-	-	-	75	-	-	7.40[i]	5.40[fg]	7.40[de]	9.40[de]	22.4[bc]	44.4[c]
-	-	-	100	-	-	14.4[bc]	10.4[ab]	9.40[bc]	7.40[f]	10.4[i]	20.4[k]
-	-	-	-	25	-	12.4[de]	6.40[ef]	9.40[bc]	15.4[a]	8.40[j]	16.4[m]
-	-	-	-	50	-	7.40[i]	7.40[de]	12.4[a]	14.4[ab]	10.4[i]	20.4[k]
-	-	-	-	75	-	12.4[de]	4.40[g]	7.40[de]	7.40[f]	20.4[de]	40.4[e]
-	-	-	-	100	-	9.40[gh]	10.4[ab]	4.40[gh]	12.4[c]	15.4[gh]	30.4[i]
-	-	-	-	-	25	8.40[hi]	11.4[a]	9.40[bc]	13.4[bc]	9.40[ij]	18.4[l]
-	-	-	-	-	50	11.4[ef]	9.40[bc]	8.40[cd]	4.40[g]	18.4[f]	36.4[g]
-	-	-	-	-	75	10.4[fg]	7.40[de]	5.40[fg]	7.40[f]	21.4[cd]	42.4[d]
-	-	-	-	-	100	11.4[ef]	9.40[bc]	8.40[cd]	8.40[ef]	14.4[h]	28.4[j]

Response of explants was calculated out of recovered explants.

Data represents the mean values of three replications, each consist of 50 explants. Mean values within each column followed by the same letter in superscript are not significantly different (P≤ 0.05: Duncan's New Multiple Range Test)

Str- Streptocycline; Ery- Erythromycin; Nor- Norfloxacin; Chl- Chloramphenicol; Oxy- Oxytetracycline; Rif- Rifampicin

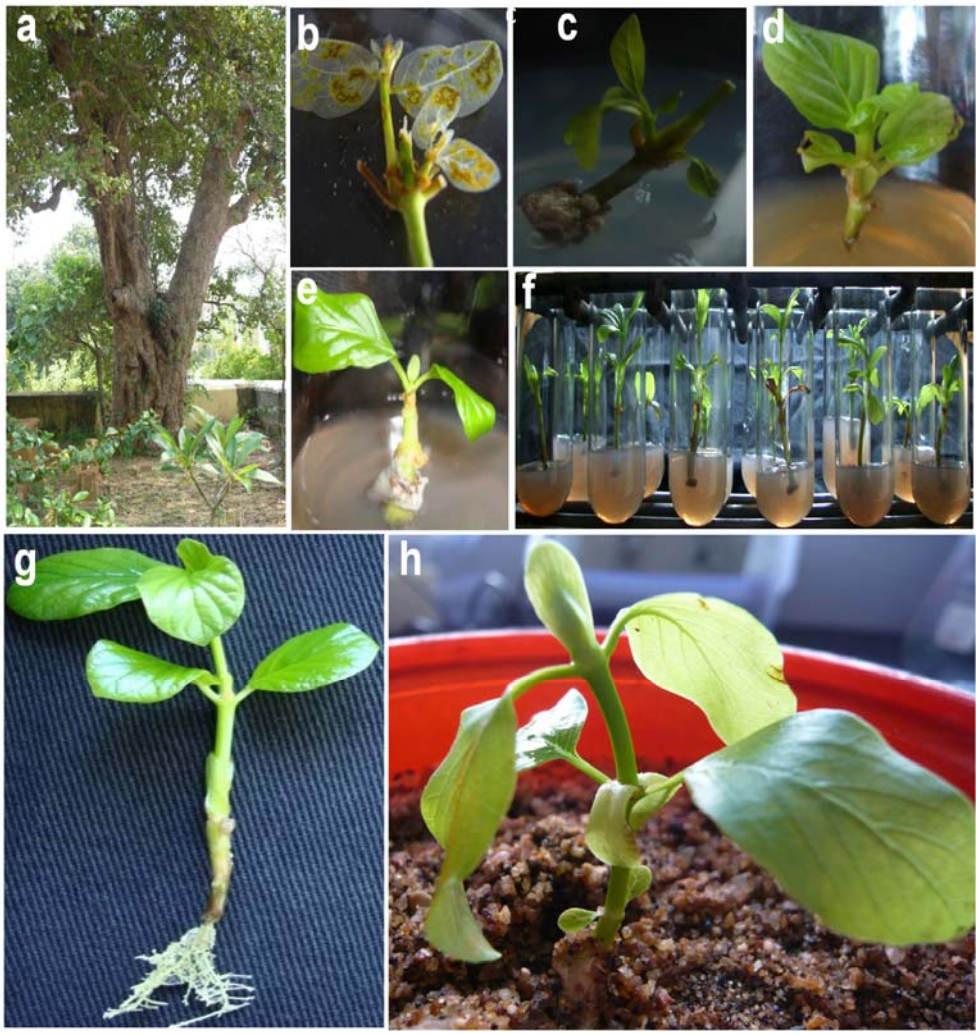

Figure 1: a) About 500 year old tree of *Anthocephalus cadamba* used as mother plant for collection of apical bud and nodal explants; b) Leaching of chlorophyll pigments from leaves and stem of young shoots when subject to disinfection with NaOCl; c &d) Regeneration of shoots from nodal and apical bud segments on MS medium supplemented with BAP (1 mg/l); e) Microshoot showing poor growth in presence of bacterial contamination; f) Regeneration of single shoots on MS medium containing BAP (1 mg/l) and erythromycin (75 mg/l); g) Ex vitro rooting of microshoots; h) Plantlets showing enlarged leaves with improved shoot growth after induction of rooting.

Efficacy of antibiotics on bacterial contamination

Effects of different antibiotics on bacterial contamination in cultured apical bud explants of *A. cadamba* is summarized (Table 5). All the explants cultured on antibiotic free medium (control) developed bacterial contamination (Fig1.e). However, those apical buds cultured on medium with different concentrations of antibiotics showed varying percentage of bacterial contamination. Of the six antibiotics tested, erythromycin (75 mg/l) recorded maximum control over bacterial contamination. All the antibiotics were ineffective at their lower concentration. However, increase in concentration of all the antibiotics from 50 – 100 mg/l improved the overall recovery of explants by minimizing the bacterial contamination. In general, though most of the cultures were observed with bacterial contamination, their growth on the medium was minimized. It was found that shoots regenerated on medium containing erythromycin, norflaxacicn, chloromphenical oxytetracycline and rifampicin at 75 mg/l grown well without any sign of

phytotoxicity even after 60 days of culture. These shoots produced healthy leaves and considerable number of such microshoots were regenerated (Fig.1f).

***Ex vitro* rooting and hardening**

Out of 35 well developed microshoots of *A. cadamba* tested for *ex vitro* rooting, about eight (22.8%) shoot were found to produce healthy roots at the end of 30 days (Fig1.g). The rooted shoots showed improved growth as evidenced by the production of 3 - 4 pairs of fresh leaves during *ex vitro* establishment. Upon further hardening, these shoots produced 4 - 5 cm long healthy shoot system with many lateral roots (Fig.1h). Whereas the unrooted shoots did not show any further growth and remained with older leaves.

The present study revealed optimum concentration of $HgCl_2$ for effective disinfection of apical bud and nodal explants using $HgCl_2$. Explants disinfected with $HgCl_2$ (0.1%) for 1 - 3 min recorded considerable number of explants for establishment of aseptic cultures. In general $HgCl_2$ was reported to be an effective disinfectants and widely used in micropropagation of several woody species such as coffee (Rajasekaran and Mohankumar 1993), cashew (D'Silva and D'Souza, 1993), tea (Rajakumar and Ayyappan, 1992; Rajasekaran and Raman, 1993), Cocoa (Mallika *et al.,* 1996), and rubber (Seneviratne and Wijesekara, 1996). The present study also indicated that $HgCl_2$ is a more effective sterilant than NaOCl for establishing apical bud and nodal explants of *A. cadamba*.

Experiments conducted using different antibiotics revealed only partial inhibition of bacterial contamination in apical bud culture of *A. cadamba.* However, the incidence and rate of bacterial growth was varied depending upon the type and concentration of antibiotic used. Among the six antibiotics tested for their efficacy, all the antibiotics were found effective only at their higher concentrations ranging from 50 – 100 mg/l. A similar results was reported when a range of antibiotics were tested for control bacterial contamination in shoot tip cultures of several woody plants (Young *et al*., 1984). In this study, rifampicin was also found effective for the control of bacterial contamination. In support of our findings, rifampicin was effective in controlling bacterial contamination in tissue culture of several species such as *Helianthus tuberosus* (Phillips *et al*., 1981), *Nicotiana plumbaginifolia* (Pollock *et al*., 1983), *Cryptocoryne* and *Cinchona* (Pierik, 1987).

Ex-vitro rooting is very popular in commercial micropropagation of important plant species. In the present study, when microshoots of *A. cadamba* was experimented for *ex vitro* rooting, high percentage (22.8%) of rooting was obtained. Though this method was well demonstrated in other plant species by several workers (Ma and Wang, 1977; Fordham *et al*., 1982; Anderson, 1978; McCown and Lloyd, 1983; Kyte and Briggs, 1979; Economou and Read, 1981 & 1986; Wong, 1981 and Ettinger and Preece 1985), in *A. cadamba*, there was no information about *ex vitro* rooting. The advantage of *ex vitro* rooting is that both root induction as well as acclimatization can be carried out simultaneously. In order to reduce *in vitro* manipulations and also to reduce the cost of production, *ex-vitro* rooting is very often adopted for commercial production of several horticultural and forest tree species. In the present study, this method has proved effective for rooting of microshoots of *A. cadamba*. It was concluded that the present protocol can be used for conservation of *A. cadamba*.

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References

Anderson, W.C. 1978. Rooting of tissue cultured rhododendrons. Comb. Proc. Int. *Plant Prop Soc.*, 28: 135-139.

Apurva, P. and Thakur, P.C. 2009. Somatic embryogenesis and root proliferation from internode of *Anthocephalus cadamba In vitro*. *Asian J. Exp. Sci*., 23 (1): 99 - 102.

Bose, T.K. and Chaudhary, B. 1991. Tropical garden plants in colour, Horticulture and allied publishers, Kolkata, India. p 13-14.

Chudnoff, M. 1984 Tropical Timbers of the world. Agricultural Handbook 607.USDA Forest service, Washington DC.466p.

D'Silva, I. and D'Souza, L. 1993. Controlling contamination and browning of *invitro* cultures of cashew. *J Plant Crops.,* 21(1): 22-29.

Economou, A.S. and Read, P.E. 1981. Effect of benzyladenine, kinetin, and 2-isopentenyladenine on *invitro* shoot formation of hardy deciduous azaleas. *Proc Plant Growth Reg Soc Am*., 8: 8-14.

Economou, A.S. and Read, P.E. 1986. Microcutting production from sequential reculturing of hardy deciduous azalea shoot tips. *Hort Sci*., 21: 137-139.

Ettinger, T.L. and Preece, J.E. 1985. Aseptic micropropagation of *Rhododendron* hybrids. *J Hort Sci*., 60: 269-274.

Fordham, I., Stimart, D.P. and Zimmerman, R.H. 1982. Axillary and adventitious shoot proliforation of Ex-bury azaleas *invitro. Hort Sci* 17: 738-739.

Kyte, L. and Briggs, B. (1979). A simplified entry into tissue culture production of *Rhododendron*. *Comb Proc Int Plant Prop Soc*., 29: 90-95.

Lopez, FR. 1966 Kaatoan Bangkal: Its nomenclature and multi-uses. For Pride Com Technical Note No.75.

Ma, S.S. and Wang, S.O. 1977. Clonal multiplication of azaleas through tissue culture. *Acta Hort* 78 : 209-213.

Mallika, V.K., Mathew, J.P., Chacko, D., Vijaya Kumar, N.K. and Nair, R.V. 1996. Induction of multiple shoots in nodal explants of fully grown trees of cocoa. *J Plant Crops* 24 (Suppl.): 503-510.

McCown, B.H. and Lloyd, G.B. 1983. A survey of the response of *Rhododendron* to *invitro* culture. *Plant Cell Tiss Org Cult*., 2: 77-85.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant*., (15): 473-497.

Patel, D. and Kumar, V. 2008. Pharmacognostical Studies of *Neolamarica cadamba* (roxb.) Bosser leaf. *Int J Green Pharm*., 2:26-27.

Phillips, R., Arnott, S.M. and Kaplan, S.E. 1981. *Plant Sci Lett*., 21:235- 240. Cited from Pollock, K., Barfield, D.G. and Shields, R. (1983). The toxicity of antibiotics to plant cell cultures. *Plant Cell Rep*., 2: 36-39.

Pierik, R.L.M. 1987. *In-vitro* Culture of Higher Plants. Martinus Nijhoff Publishers, Dordrecht, Netherlands, p.73.

Pollock, K., Barfield, D.G. and Shields, R. 1983. The toxicity of antibiotics to plant cell cultures. *Plant Cell Rep*. 2: 36-39.

Rajakumar, R. and Ayyappan, P. 1992. Micropropagation of *Camellia sinensis* (L.) O. Kuntze. *J Plant Crops* 20 (Suppl.): 252-256.

Rajasekaran, P. and Mohankumar, P. (1993). Mass clonal propagation of coffee *invitro*. *J Plant Crops*., 21 (Suppl.): 301-306.

Rajasekaran, P. and Raman, K. 1993. Propagation of tea by *invitro* culture of nodal explants. *J Plant Crops* 21(1): 8-14.

Santapan H. and Henry, A.N. 1973. A dictionary of Flowering Plants in India, Council of Scientific Industrial Research Publication, New Delhi.

Seneviratne, P. and Wijesekara. 1996. The problem of phenolic oxidation in *in-vitro* culture s of mature *Hevea brasiliensis*. *J Plant Crops* 24: 54 - 62.

Wong, S. 1981. Direct rooting of tissue cultured rhododendrons into an artificial soil mix. *Comb Proc Int Plant Prop Soc* 31: 36-39.

Young, P.M., Hutchins, A.S. and Canfield, M.L. 1984. *Plant Science Lett*., 34: 203-209. Cited from Zoe, A.W. and Power, J.B. (1989). Elimination of systemic contamination in explant and protoplast cultures of rubber (*Hevea brasiliensis* Muell. Arg.). *Plant Cell Rep*., 7: 622-625).

Zabala N.Q. and Manarpaac, V. 1968 The effects of fertilization on growth of *Kaatoan bangkal* seedlings. *Philipp Lumber*., 14:36-41.

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