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Optimization of *in vitro* culture establishment in *Aglaonema* sp. by employing shoot tips and nodal segments as explants

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Abstract
All over the world, *Aglaonema* are acclaimed as one of the most popular indoor ornamental plants. Vegetative propagation through stem cuttings is relatively slow and can carry diseases from mother stock to cuttings. Micro-propagation is order of the day for producing large scale quality, disease free and physiologically uniform genetic stock in any plant species. However, establishment of *in vitro* axenic cultures in *Aglaonema* is relatively difficult as endogenous pathogens harbour in their vascular tissue. This experiment was under taken to standardize efficient pre-treatment and surface sterilization protocols for aseptic culture establishment by using shoot tips and nodal segments as explants in *Aglaonema* sp. at Dr. YSRHU-Horticultural Research Station, Kovvur during the year 2022-23. Experiment was laid in factorial completely randomized design. Among the different treatments, highest axenic culture establishment was recorded in both the explants treated with Carbendazim 0.2% + Mancozeb 0.2% + Plantomycin (200 ppm) + Tween-20 for two hours as pre-treatment followed by series of treatments with 4% NaOCl for 30 min. followed by 0.1% $HgCl_2$ for 15 min. followed by 80% Ethanol for 1 min. followed by treating the buds with Cefotaxime 200 ppm for 30 min. as a surface sterilization treatment. Among the different growth regulators tested, MS media supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA recorded highest culture establishment, number of shoots per explant in minimum number of days.

Keywords: Araceae, propagation, meristem tissue, axenic culture, survival, micro-propagation

Introduction
The term ornamental plants refer to all plants with ornamental value which generally have beautiful flowers, foliage or special plant architectures. Ornamental plants are both indoor and outdoor plants, which aid to improve the aesthetics of our homes, offices and are best option to make our planet greener. These are natural air purifiers and improve the aesthetics of our surroundings. Among the indoor ornamental plants, *Aglaonema* is highly popular and first choice for indoor landscapists. *Aglaonema* have been grown as luck bringing ornamental plants in Asia for centuries. They were introduced to the West in 1885, when they were first brought to the Royal Botanic Garden, Kew. They have been cultivated, hybridized and bred into a wide array of new cultivars that have been released continuously to maintain the popularity of plants (Chen *et al*., 2002) [7]. Their preference for relatively low to bright or indirect light levels helps them to thrive under artificial lighting in indoor conditions. They are usually grown under 75 to 90% shade conditions commonly seen in waiting areas, lobbies, office buildings, etc. It consists of valuable cultivars of variegated foliage plants renowned for their beautiful shape, variation in leaf colour, size, markings, tolerance to drought and low relative humidity (Yeh *et al*., 2007) [28]. These are also popular in public and industrial environments because it is effective at reducing typical household air pollutants like formaldehyde and benzene, according to the NASA clean air study (Wolverton *et al*., 1989) [26]. These indoor plants are quite popular these days, particularly after the COVID-19 epidemic.

Aglaonema is a genus of flowering plants also known as chinese evergreen, a monocot which belongs to Araceae family and native of tropical and subtropical regions of Asia and New Guinea (Chen *et al*., 2003) [6]. The word *Aglaonema* is derived from two greek words 'aglos' meaning bright or clear and 'nema' meaning a thread in reference to the stamens. It has 21 species. The basic chromosome number could be x = 6, with subsequent polyploidy in many

cases. These plants are famous as a foliage ornamental planta due to its attractive foliage, easiness to grow and tolerance to low light conditions and low relative humidity (Hussein, (2002), Chen *et al*., (2002)) [17, 5]. Sexual reproduction is challenging since flowers don't bloom simultaneously, pollen has a short life span and due to segregating population. Hence, most of *Aglaonema* sp. have been propagated by stem cuttings from nodes or shoot basal division as the basic method (Barakat and Gaber, 2018) [2]. However, propagation through cuttings has a relatively slow rate of multiplication and can carry diseases from stock plants to cuttings. Furthermore, some *Aglaonema* cultivars may harbour endogenous pathogens in their vascular tissue (Chase, 1997) [4], rendering cuttings a potential vehicle for the transmission of disease. Every year, breeders develop new types/varieties/hybrids, and there is a need to introduce these new forms to the landscape sector through rapid multiplication techniques.

Micro-propagation techniques are advanced vegetative propagation tool for producing a large amount of uniform, pathogen-free propagules in a short period of time and limited space. Thereby it is feasible to produce stock plants in smaller greenhouses and provides growers a source of healthy tissue-cultured plantlets throughout the year (Chen and Henny, 2008) [8]. However, the difficulty of establishing aseptic culture (Chen and Yeh, 2007) [6] and low rate of shoot multiplication (Zhang *et al*., 2004) [29] are major hindrances in its *in vitro* multiplication. Establishment of *in vitro* axenic cultures in this crop is relatively difficult as endogenous pathogens harbour in their vascular tissue. Moreover, lack of sufficient information on reproducible *in vitro* protocol is another major limiting factor in this crop (Mariani *et al*., 2011) [22]. As a result, the current work aims to standardise effective pre-treatment and surface sterilisation methods along with successful aseptic culture establishment in *Aglaonema* sp.

Materials and Methods

Present experiment was carried out to standardize aseptic culture establishment in *Aglaonema* sp. during the period from 2022 to 2023 at Tissue culture laboratory, Dr. YSRHU-Horticultural Research Station, Kovvur supported by College of Horticulture, Dr. YSRHU, Venkataramannagudem, West Godavari, Andhra Pradesh.

Plant material

Disease free, healthy grown, three months old *Aglaonema* variety **'**Hungary' potted plants were selected as mother plants. These plants were maintained in a 50% green shade net house with all the recommended crop management practices. Irrigation was stopped for one week before collecting nodal segments and shoot tips as explants and sprayed the plants with Blitox 3.0 g L^{-1} + Streptocycline 1.0 g L^{-1} to reduce microbial contamination. Early in the morning, shoot tips and nodal segments were collected and prepared into 1.5 to 2.5 cm segments by removing the leaves, attaching leaf petiole sheaths, and exposing the dormant buds on nodes.

Pre-treatment and surface sterilization

The prepared nodal and shoot tip segments were thoroughly washed under running tap water for 1 hour to remove the dust and other surface contamination. Explants without any mechanical visible damage were collected and treated with different combinations and concentrations of Carbendazim, Mancozeb and Plantomycin in different durations. After pre-treatment the explants were thoroughly rinsed with autoclaved distilled water thrice in laminar air flow chamber. Later on the pre-treated explants were dipped in 4% sodium hypochlorite (NaOCl) solution followed by 0.1% $HgCl_2$ and 80% ethanol treatments. In each step, after chemical surface sterilization, the explants were rinsed thrice with sterile distilled water, to lower the toxic effects of chemicals used and became ready for *in vitro* culture initiation.

After sterilization, the explants were dried on sterilized tissue paper, removed the dried leaf sheaths, petioles and gave a sharp cut in the lower side of explants to expose the fresh tissue. Then the sterilized explants were transferred to the culture media prepared in test tubes by pressing with the sterilized forceps gently to get firm contact of explant with the media. The test tubes were closed with poly propylene caps and wrapped with cling film.

After adjudging the best pre-treatment and surface sterilization, the explants were transferred to Murashiage & Skoog media (1962) [23] with different concentrations of BAP (0, 1.0, 2.0 and 3.0 mg L^{-1}) in combination with NAA (0.5 mg L^{-1}) solidified with agar-agar 7.0 g L^{-1}. The pH of the culture media adjusted to 5.8 before adding the gelling agent and then sterilized at 121 °C for 18 min.

Observations were recorded for nature of contamination, per cent fungal contamination, per cent bacterial contamination, per cent explant mortality other than microbial contamination, total explants mortality, per cent phenol exudation, per cent explant survival, culture establishment index, number of shoots per explant, number of days taken for response after 25 days of culture initiation.

In this experiment, four replications were used in each treatment. Each replication contains eight test tubes and each test tube contains 15 ml media with single explant. The glassware and culture media were prepared by autoclaving as mentioned earlier. After inoculation, the explants were incubated in growth chamber at 26±1 °C temperature under 16 hr daily light and 8 hr dark illumination by a LED light with intensity of 3000 Lux.

Statistical analysis

The data collected for all the parameters involved in this study were subjected to statistical analysis using OPSTAT as per the design adopted in corresponding experiments. The analysis of variance (ANOVA) was performed as per the outlines described by Panse and Sukhatme (1985) [30]. Statistical significance was tested by employing F table value at 5% level of significance. The critical difference value at 5% level of significance was calculated wherever differences among treatments means were found significant. The appropriate standard errors of mean (SE m) were calculated in each case.

Results and Discussions

Pre-treatment

A perusal of data presented in Table. 1, showed a significant difference among pre-treatments with combination of fungicide, bactericide and surfactant at various concentrations and durations of exposure.

Among explants, nodal segments showed least fungal (30.6%), bacterial contaminations (16.5%), total explant mortality (47.1%) and highest explant survival (52.8%).

While highest fungal (33.2%), bacterial contaminations (18.3%), total explant mortality (51.1%) and least explant survival (48.8%) was shown by shoot tips. There was no significant difference found among explants with respect to all the parameters noted.

Significant reduction in fungal (18.5%), bacterial contaminations (10.1%), total explant mortality (28.7%) with higher explant survival (71.3%) was noted when the explants were pre-treated with Carbendazim (0.2%) + Mancozeb 0.2% + Plantomycin 200 ppm +Tween-20 for 120 minutes (P_5). Where as maximum fungal (59.6%), bacterial contaminations (27.9%), total explant mortality (87.6%) and minimum explant survival (12.3%) was noted when the explants were washed with distilled water (control) for 60 minutes (P_1).

Among interactions, nodal segments treated with Carbendazim (0.2%) + Mancozeb 0.2% + Plantomycin 200 ppm + Tween-20 for 120 minutes (P_5) renowned with minimum fungal (17.4%), bacterial contaminations (9.2%), total explant mortality (26.6%) with higher explant survival (73.3%). Significant difference among interactions was not observed in the present study conducted with respect to the parameters considered.

Most of the biological organisms harbour varied types of microbial contaminations, both exogenous and endogenous in nature. By employing suitable fungicides and bactericidal pre-treatments one can easily eliminate the surface contaminations. In the present study, pre-treatment of nodal segments and axillary nodes with Carbendazim (0.2%) + Mancozeb (0.2%) + Plantomycin (200 ppm) + Tween-20 (1%) for two hours had exhibited superior performance in controlling both fungal and bacterial contaminations without causing any toxicity to explants.

Carbendazim is a highly effective systemic fungicide that controls several fungal diseases by interfering with DNA synthesis in pathogenic fungi. Mancozeb is a contact fungicide which disrupts cytoplasm and mitochondria thereby reducing vital enzymatic activity in fungus thus damaging energy metabolism ultimately leading to death of fungus. The efficacy of these compounds *i.e.* carbendazim and mancozeb in controlling microbial contamination were also reported by Bharadwaj *et al.,* (2006) [3] and Kumari *et al.,* (2013) [21] in rose, Kumar *et al*., (2017) [20] in Marigold and plantomycin efficacy in reducing bacterial growth was described by Yenjerappa *et al*., (2014) [27] in pomegrante.

Higher doses of pre-treatment chemicals with longer periods were shown to be more efficient in lowering overall explant mortality across the various combinations of concentrations and durations tested. The efficiency of pre-treatment combination was greater when both bactericide and fungicide were used compared to those only either of them were used (Prathyusha *et al.,* 2021) [24]. This might be due to the synergistic effect of carbendazim and mancozeb with antibiotics. These results are in close conformity with the reports of Fang *et al*., (2013) [14].

Similarly, Abass *et al*. (2016) [1] reported the use of antifungal solution (Moncut) @ 3 g L^{-1} along with few drops of Tween-20 for 30 min. followed by immersion of explants in Salvon Salvon @ 15% for 10 min. to reduce the fungal contamination in *Aglaonema commutatum* plants. Mariani *et al*. (2011) [22] suggested the use of Antracol (active compound: 70% propineb) as fungicide for 30 min. for pre-treatment of axillary shoots of *Aglaonema*. However, in *Aglaonema* sp. For the first time we have reported the use of carbendazim and mancozeb as pre-treatment chemicals for significant reduction of fungal contaminations in *in vitro* cultures of *Aglaonema*.

Surface Sterilization

Surface sterilization of explants with different combinations at various durations had significant effect on explant survival as shown in table-2. There were no significant differences observed among the two explants evaluated in the present study. Among the surface sterilization treatments, highest per cent explant survival (75.3%) with minimum fungal contamination (21.3%), bacterial contaminations (3.3%), total explant mortality (24.6%) was registered when explants treated with NaOCl 4.0% (30 min.) followed by 80% ethanol (1 min.) followed by $HgCl_2$ 0.1% (15 min.) followed by Cefotaxime 200 ppm (30 min.) (S_7).

The interactions between explants and surface sterilization treatments revealed that, nodal segments treated with NaOCl 4.0% (30 min.) followed by 80% ethanol (1 min.) followed by $HgCl_2$ 0.1% (15 min.) followed by Cefotaxime 200 ppm (30 min.) (S_7) exhibited maximum survival (75.6%) with minimum fungal contamination (21.8%), bacterial contaminations (2.5%), total explant mortality (24.3%). There was no significant difference found among interactions regarding the parameters recorded.

Axenic culture establishment was a crucial and important step for the successful development of *in vitro* commercial multiplication protocol of any species. Explants such as nodal segments, shoot tips, leaves, petioles, florets etc. collected from field grown plants possessed with wide range of microorganisms which need to be eliminated by efficient surface sterilization procedure for *Aglaonema* has been reported by different workers Chen and Yeh, (2007) [7]; Fang *et al*., (2013) [14]; Abass *et al*., (2016) [1]; Barakat and Gaber, (2018) [2]; El-Gedawy and Hussein, (2022) [10] but independent surface sterilization protocol in combination with pre-treatment need to be developed depending upon the hardness of tissue, genotype, availability of sterilizing agent and local regulations.

These results were in conformity with the results obtained by earlier workers Abass *et al*., (2016) [1]; Barakat and Gaber, (2018) [2]; El-Gedawey and Hussein, (2022) [10] for shoot tips and nodal segments as explants in *Aglaonema*. To the contrary, some of earlier researchers claimed that NaOCl was solely used as surface sterilant Chen and Yeh, (2007) [7]; Mariani *et al*., (2011) [22] and Kaviani *et al*., (2019) [19] and others treated explants with only $HgCl_2$ as disinfectant Juxian *et al*. (2013) [18]. Abass *et al*., (2016) [1] suggested using 0.3% mercuric chloride solution for 15 min. in shoot tips obtained from *Aglaonema* commutation plants. Likewise, Fang and Hsu, (2012) [13] reported the combination of Gentamycin, tetracycline and chloramphenicol for successful reduction of bacterial growth in *Aglaonema in vitro* cultures.

Culture Initiation

As per the results envisaged in Table 3, all the media fortified with growth regulators showed considerably higher establishment over the control *i.e.* modified MS medium devoid of growth regulators (control) (T_1).

Among the explants, nodal segments recorded earliest shoot emergence (9.8) with highest culture response (70.7%), number of shoots per explant (1.5) and culture establishment index (111.8) over shoot tips as explants.

Among the different growth regulator treatments, maximum culture response (81.1%) with highest number of shoots (2.0), earliest with respect to days to shoot emergence (7.1) and maximum culture establishment index (158.9) was shown by explants cultured in MS media added with 2.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA (T_3). Explants cultured in MS media devoid of growth regulators (control) recorded least culture response (58.63%) with least number of shoots per explant (1.07), more number of days to shoot emergence (17.50) and least culture establishment index (62.73). The interaction between explants and plant growth regulators was non-significant.

The regeneration potentiality of the explant varied with type of explant, growing conditions and its response to the *in vitro* treatments. In the present studies, among the two explants, better culture establishment and response was noticed in axillary buds over shoot tips as explants. When the axillary buds were used as explants, maximum culture establishment was observed in relatively less number of days compared to shoot tips as explants. This might be due to presence of 2 to 4 dormant axillary buds present on each nodal segment instead of single apical meristem in shoot tip as explant. Additionaly, juvenile leaves firmly overlap the apical meristem near the shoot tip, which may have hampered the bud`s ability to sprout quickly. The sprouting of several axillary buds in a short period of time may possibly be related to the elimination of apical dominance in axillary nodes in the process of culture initiation. Axillary bud has been used as starting material for commercial multiplication of *Aglaonema* by several workers earlier also Chen and Yeh, (2007) [7]; Mariani *et al*., (2011) [21]; Junxian *et al*., (2013) [19]; Barakat and Gaber, (2018) [2] and El-Gedawey and Hussein, (2022) [10]

In the current study, both the explants cultured on MS media supplemented with BAP 2.0 mg L^{-1} was recorded superior performance with respect to highest explant response in less number of days with more number of shoots per explant over all growth regulator combinations. Similar results were reported by earlier workers Junxian *et al*., (2013) [18]; Barakat and Gaber, (2018) [2] and Kaviani *et al.,* (2019) [19]. In contrast to this, considerable number of workers also reported the use of TDZ as growth regulator Chen and Yeh (2007) [7]; Mariani *et al*., (2011) [22]; Fang *et al.,* (2013) [14]; El-Mahrouk *et al*., (2016) [12] and El-Gedawey and Hussein, (2022) [10]. The roles of cytokinin and auxin in micropropagation are well understood, and the combination of suitable auxins and cytokinins can have a synergistic impact to produce the greatest morphogenic response.

The effectiveness of cytokinins in stimulating cell division, shoot bud morphogenesis, breaking apical dominance, and releasing growth of lateral buds may be responsible for current research finding George *et al*., (2008) [16]. Numerous studies showed that BA is more effective than other cytokinins at releasing axillary buds from apical dominance in other cytokinins at releasing axillary buds from apical dominance in other members of the Araceae family such as Diffenbachia El-Mahrouk *et al.,* (2006) [11] and Spathiphyllum Dewir *et al.,* (2006) [9]. In their studies, Barakat and Gaber, (2018) [2] suggested to use cytokinins along with auxin for obtaining the maximum number of shoots per explant in comparison with treatments with BAP alone.

Table 1: Effect of different pre-treatment methods on nature of microbial contamination, mortality and survival of explants in *Aglaonema* sp.

Pretreatments		Fungal contamination (%)			Bacterial Contamination (%)			Total explants mortality (%)			Survival (%)		
		Shoot tip	Nodal segments	Mean	Shoot tip	Nodal segments	Mean	Shoot tip	Nodal segments	Mean	Shoot tip	Nodal segments	Mean
T_1	Control (Distilled water wash and best surface sterilization treatment)	59.74 (50.66)	59.62 (50.60)	59.66 (50.61)	28.49 (32.16)	27.44 (31.52)	27.96 (31.84)	88.23 (70.06)	87.10 (69.72)	87.66 (69.89)	11.67 (19.89)	12.90 (20.23)	12.33 (20.06)
T_2	Carbendazim (0.1%) + Mancozeb (0.1%) + Plantomycin (200 ppm) + Tween-20 for 60 minutes	33.25 (35.15)	29.02 (32.57)	31.15 (33.86)	19.87 (26.30)	16.00 (23.20)	17.95 (24.75)	53.16 (46.85)	45.00 (42.07)	49.08 (44.46)	46.83 (43.11)	55.00 (47.89)	50.91 (45.50)
T_3	Carbendazim (0.2%) + Mancozeb (0.2%) + Plantomycin (200 ppm) +Tween-20 for 60 minutes	28.85 (32.33)	27.76 (31.64)	28.31 (31.98)	19.04 (25.60)	15.96 (23.36)	17.50 (24.48)	45.70 (42.56)	43.70 (41.31)	44.70 (41.90)	54.30 (47.45)	56.30 (48.65)	55.30 (48.05)
T_4	Carbendazim (0.1%) + Mancozeb (0.1%) + Plantomycin (200 ppm) + Tween-20 for 120 minutes	24.42 (29.56)	19.44 (26.11)	21.93 (27.84)	13.31 (20.93)	13.89 (21.83)	13.60 (21.38)	37.76 (37.88)	33.30 (35.20)	35.53 (36.54)	62.23 (52.07)	66.70 (54.76)	64.46 (53.41)
T_5	Carbendazim (0.2%) + Mancozeb (0.2%) + Plantomycin (200 ppm) + Tween-20 for 120 minutes	19.76 (26.25)	17.41 (24.59)	18.58 (25.43)	10.98 (19.18)	9.28 (17.67)	10.13 (18.43)	30.73 (33.50)	26.66 (31.06)	28.70 (32.28)	69.26 (56.45)	73.33 (58.90)	71.30 (57.68)
Mean		33.20 (34.79)	30.65 (33.09)		18.34 (24.83)	16.50 (23.51)		51.11 (46.16)	48.00 (43.87)		48.88 (43.80)	52.84 (46.09)	
		Factor A	Factor B	A X B	Factor A	Factor B	A X B	Factor A	Factor B	A X B	Factor A	Factor B	A X B
SE (m)		-	1.6	-	-	1.704	-	-	1.974	-	-	1.974	-
CD		NS	4.754	NS	NS	5.062	NS	NS	5.863	NS	NS	5.863	NS

Table 2: Effect of different surface sterilization methods on nature of microbial contamination, mortality and survival of explants in *Aglaonema* sp.

	Surface Sterilization	Fungal contamination (%)			Bacterial Contamination (%)			Total explants mortality (%)			Survival (%)		
		Shoot tip	Nodal segments	Mean	Shoot tip	Nodal segments	Mean	Shoot tip	Nodal segments	Mean	Shoot tip	Nodal segments	Mean
S_1	Control (Distilled water wash followed by best pre-treatment)	64.48 (53.40)	64.07 (53.15)	64.27 (53.28)	28.83 (32.45)	28.23 (32.06)	28.53 (32.26)	93.31 (74.98)	92.30 (73.86)	92.80 (74.42)	6.68 (14.97)	7.70 (16.10)	7.19 (15.53)
S_2	NaoCl (4%) for 15 min.+ $Hgcl_2$ (0.1%) for 10 min + 80% Ethanol for 1 min.	33.29 (35.17)	29.33 (32.76)	31.31 (33.96)	20.06 (26.58)	21.33 (27.47)	20.69 (27.03)	53.35 (46.91)	50.67 (45.36)	52.01 (46.13)	46.64 (43.05)	49.33 (44.60)	47.9 (43.82)
S_3	NaoCl (4%) for 30 min.+ $Hgcl_2$ (0.1%) for 10 min + 80% Ethanol for 1 min.	31.25 (33.97)	28.33 (32.07)	29.79 (33.02)	18.75 (25.64)	15.44 (23.01)	17.09 (24.33)	50.00 (44.98)	43.77 (41.35)	46.88 (43.16)	50.00 (44.98)	56.22 (48.60)	53.11 (46.79)
S_4	NaoCl (4%) for 15 min.+ $Hgcl_2$ (0.1%) for 15 min + 80% Ethanol for 1 min.	28.83 (32.45)	21.81 (27.81)	25.32 (30.13)	15.59 (23.15)	18.01 (25.06)	16.81 (24.10)	44.42 (41.78)	39.83 (39.10)	42.13 (40.44)	55.57 (48.18)	60.17 (50.85)	57.87 (49.51)
S_5	NaoCl (4%) for 30 min.+ $Hgcl_2$ (0.1%) for 15 min+80% Ethanol for 1 min.	24.22 (29.36)	24.00 (29.27)	24.11 (29.31)	13.37 (21.43)	12.00 (20.08)	12.68 (20.75)	37.59 (37.77)	36.00 (36.85)	36.79 (37.31)	62.40 (52.18)	64.00 (53.10)	63.20 (52.64)
S_6	NaoCl (4%) for 15 min.+ $Hgcl_2$ (0.1%) for 10 min + 80% Ethanol for 1 min. + Cefotaxime (200 PPM) for 30 min.	22.14 (28.03)	23.10 (28.71)	22.62 (28.37)	8.90 (17.12)	8.93 (17.32)	8.92 (17.22)	31.05 (33.84)	32.03 (34.45)	31.54 (34.14)	68.95 (56.12)	67.96 (55.51)	68.45 (55.81)
S_7	NaoCl (4%) for 30 min.+ $Hgcl_2$ (0.1%) for 15 min + 80% Ethanol for 1 min. + Cefotaxime (200 PPM) for 30 min.	20.83 (27.09)	21.81 (27.81)	21.32 (27.45)	4.16 (9.64)	2.56 (7.53)	3.36 (8.59)	25.00 (29.87)	24.38 (29.51)	24.69 (29.69)	75.00 (60.09)	75.61 (60.45)	75.30 (60.27)
Mean		32.15 (34.21)	30.35 (33.08)		15.66 (22.29)	15.21 (21.79)		47.82 (44.30)	45.57 (42.91)		52.1 (45.65)	54.4 (47.03)	
		Factor A	Factor B	A X B	Factor A	Factor B	A X B	Factor A	Factor B	A X B	Factor A	Factor B	A X B
SE (m)		-	0.92	-	-	1.44	-	-	1.02	-	-	1.02	-
CD at 5%		NS	2.69	NS	NS	4.21	NS	NS	2.97	NS	NS	2.97	NS

Table 3: Effect of BAP and NAA on *in vitro* culture establishment (%), number of shoots per explant, days to shoot emergence, shoot length, culture establishment index in *Aglaonema* sp. using shoot tips and axillary nodes as explants

Treatment		Culture response (%)			No. of shoots per explant			No. of days to shoot emergence			Culture establishment index		
		Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean	Shoot tip	Nodal segment	Mean
T_1	MS media (Devoid of growth regulators)	56.14 (48.50)	61.12 (51.40)	58.63 (49.95)	1.07	1.07	1.07	18.00	17.00	17.50	60.06	65.39	62.73
T_2	MS + BAP 1.0 mg L^{-1} + NAA 0.5 mg L^{-1}	64.44 (53.39)	66.66 (54.74)	65.55 (54.06)	1.40	1.33	1.37	9.47	7.93	8.70	90.21	88.65	89.43
T_3	MS + BAP 2.0 mg L^{-1} + NAA 0.5 mg L^{-1}	79.12 (62.83)	83.19 (65.92)	81.15 (64.30)	2.20	1.73	1.97	7.20	7.07	7.13	174.06	143.91	158.99
T_4	MS + BAP 3.0 mg L^{-1} + NAA 0.5 mg L^{-1}	73.2 (58.97)	72.19 (58.20)	72.71 (58.58)	1.47	2.07	1.77	7.73	7.27	7.50	107.66	149.43	128.55
	Mean	68.2 (55.9)	70.79 (57.56)		1.53	1.55		10.60	9.82		107.66	111.85	
		Factor A	Factor B	A X B	Factor A	Factor B	A X B	Factor A	Factor B	A X B	Factor A	Factor B	A X B
	SE(m)	0.85	1.21	1.71	0.04	0.06	0.09	0.23	0.33	0.47	4.05	5.73	8.10
	CD at 5%	NS	3.66	NS	NS	0.19	0.27	0.71	1.00	NS	NS	17.33	24.51

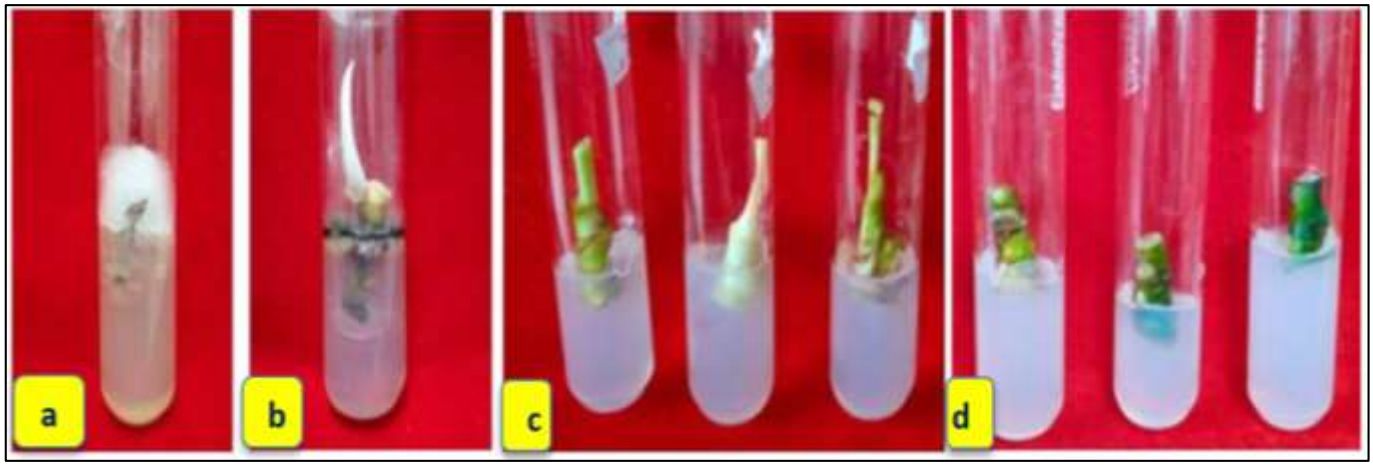

Fig 1: *Aglaonema* cultures a) fungal contamination; b) bacterial contamination; c &d) shoot tips and nodal segments as explants at the time of initiation

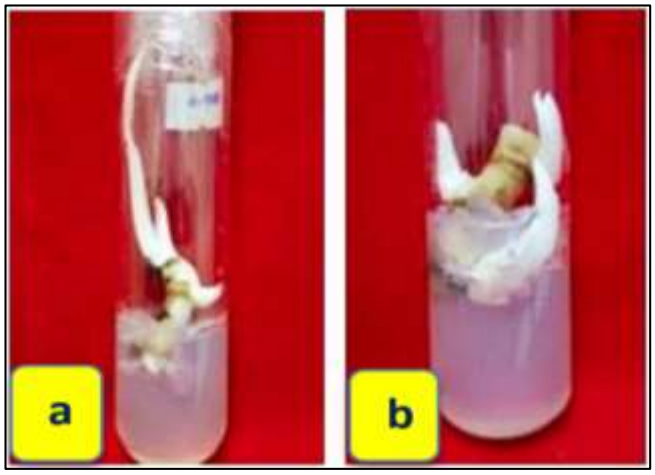

Fig 2: Regenerated shoots from (a) shoot tips and (b) nodal segments of *Aglaonema* on MS medium supplemented with 2.0 mg L^{-1} BAP + NAA 0.5 mg L^{-1}

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