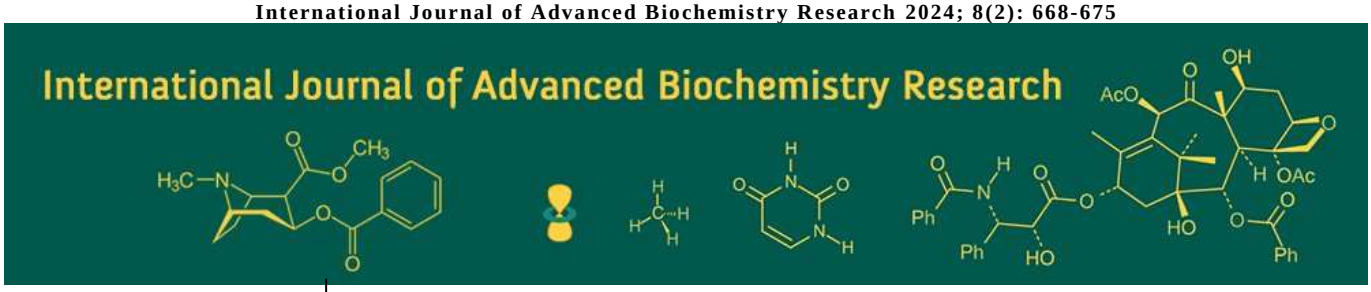

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Advances in micropropagation of *Aglonema* var. Red valentine

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Abstract
Aglaonema, a popular ornamental plant valued for its vibrant foliage and aesthetic appeal. This study aimed to establish an efficient protocol for *in vitro* propagation of *Aglaonema* variety Red valentine using tissue culture techniques. The experiment involved the establishment of sterile cultures from nodal explants. Murashige and Skoog (MS) medium supplemented with various concentrations of plant growth regulators used to stimulate organogenesis. Surface sterilization of explants with carbendazim (0.2%) + Plantomycin (200 mg/l) for 30 minutes, along with Ethanol 70% for 1 minute, Sodium hypochlorite 10% for 15 minute, and Mercuric chloride 0.3% for 15 minute, showed the highest aseptic cultures (88.67%). Murashige and Skoog's medium supplemented with 3.0 mg/l Benzyl-amino-purine resulted in 84% of bud sprouting. Highest multiplication ratio (1: 2.53) obtained on Murashige and Skoog's medium supplemented with 6 mg/l Benzyl-amino-purine and 2 mg/l of Kinetin. Maximum rooting (95%) was observed in medium supplemented with 2.0 mg/l Indole-3-butyric acid and 0.5 mg/l Naphthalene acetic acid. Plantlets were successfully hardened in 100% of Cocopeat in greenhouse.

Keywords: *Aglaonema*, organogenesis, micropropagation, hardening

Introduction
Aglaonema, commonly known as Chinese evergreen, is a popular ornamental plant prized for its attractive foliage and adaptability to indoor environments, which commonly known as aroids important in interior landscaping [1]. *Aglaonema* genus comprised 21 species which inhabit humid and heavily shaded forests of many territories of Asia [2]. *Aglaonema* easiness to grow and tolerance to low light conditions and low relative humidity [3].
The rooting of its cuttings and division of basal shoots are the basic methods of propagation since non-simultaneous flowering and short life span of the pollen make sexual reproduction difficult [4]. Adoption of *in vitro* propagation has reduced the time-spanned for plant multiplication [5]. The process of *in vitro* micropropagation involves the manipulation of plant cells, tissues, and organs on a nutrient-rich agar medium supplemented with specific plant growth regulators. The importance of this research work is to understanding and examining the proper amount of different concentrations of plant growth regulators in medium for the proper growth of the *Aglaonema* plantlets Red valentine.

Materials and Methods
Plant Materials
Aglaonema var. Red valentine was used for research study. The study was conducted in plant tissue culture laboratory of Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidhyapeeth, Dapoli, Ratnagiri, Maharashtra state, India. Explants were collected from the ornamental nursery of College of Horticulture, Dr. Balasaheb Sawant Krishi Vidhyapeeth, Dapoli. Healthy and disease-free planting materials were selected as the initial source for culture establishment. Typically, young shoots, nodal segments, or meristematic tissues were chosen as explants due to their high regenerative capacity and contains low contaminants as compare to mature explants.

Position and size of nodal segments
Surface sterilization treatment plays an important role in establishment of *in vitro* cultures. Sterilants not only removes microorganisms but also causes phyto-toxic effect in plant tissue.

After sterilization, immature and small size explants turn to brown and simultaneously dead. Large size explants, contains more microbial count, tolerate harsh sterilants treatment but hinder bud sprouting. Nodal segments and apical tips were collected and brought to laboratory. Nodal position counted from apex to base and these explants cut into various sizes and arranged according to their nodal position. The bud break frequency and occurrence of contamination in respect to their size and position were recorded.

Surface sterilization of explants

Firstly explants were kept under running tap water for 30 min. Then explants were transferred inside the laminar airflow, inside a sterilized jars containing 0.2% Carbendazim and treat for 30-45 min. After that, explants were washed with sterilized distilled water at least 3-4 times. The explants were treated with 70% isopropanol for about 1 min then they were washed using sterilized distilled water about 2-3 times. Next the explants were treated with sodium hypochlorite solution and then washed 2-3 times with sterilized distilled water. Then explants were treated with $HgCl_2$ solution, again washed with sterilized distilled water about 3-5 times (Table-1). After the surface sterilization, explants were removed from the bottle jar and placed on sterilized tissue paper to dry. Note that during washing of explants with sterilized distilled spend 5 min in each wash.

Culture Medium preparation

Many researchers use MS medium [6] and also find advantages in shoot multiplication of many *Aglaonema* species like *A.* red valentine or *A.* Red widuri [7]. MS medium contains various required, high salts concentration of elements and reported best for *in vitro* propagation by many researchers. In all experiments, full strength MS medium were used for *Aglaonema* micropropagation. Required stock solutions of MS medium and plant growth regulators prepared well in advance and stored in refrigerator. During medium preparation there stock solutions bring to room temperature and all stock solution will mix one by one and final volume adjusted by sterilized distilled water. Required pH of the medium was adjusted with the help of NaOH and/or HCl before autoclaving. After adjusting pH, equal volume boiled medium dispensed in culture glass jar bottles. Medium autoclaved at temperature of 121 °C for 20 min. Autoclaved medium stored in the dark at 24±2 °C and used after one week of sterilization. MS-medium supplemented with various different concentrations of plant growth regulators for bud sprouting, shoot multiplication, rooting and their results on proliferation of *Aglaonema* were recorded.

Experimental Conditions

The culture chamber/growth rooms in which the experiments were conducted was kept at a constant temperature of 25± °C, and a 16/8-hour light/dark cycle was used to provide consistent illumination of light (1600 Lux) using white fluorescent lights.

Hardening of *in vitro* plantlets:

Healthy well grown plantlets obtained after 4 to 5 weeks of inoculation on rooting medium were taken out from jars and washed thoroughly with water to remove traces of adhere medium. These plantlets were treated with carbendazim (1%) solution for 30 minute and transplanted in different potting mixtures and their observations were recorded. The plantlets were kept in shade (in polyhouse) for one week and then transferred to partial-sunlight in green shade-net house. Thereafter, plantlets nurtured inside for 2 months.

Statistical analysis

The data recorded from the experiments were conducted according to completely randomized design (CRD). Each experiment was replicated three times and each replication contains 20 test samples. The goal of the study was to find a significant difference between the treatment means. On the basis of critical difference, the treatment means were classified as significant or non-significant (CD) [8].

Results and Discussion

Establishment of asceptic cultures

In present investigation, ten different treatments were used for sterilization. T1 to T9 showed low percentages of aseptic culture, with fungal and bacterial contaminants being the major occurrence. As the concentration of $HgCl_2$ increased an increase in aseptic culture observed. Treatment T10, which utilized a combination of carbendazim (0.2%) and Plantomycin (200 mg/l) for 30 mins along with Ethanol (70%) 1 min, NaOCl (10%) 15 mins , and $HgCl_2$ (0.3) 15 mins, showed the highest aseptic culture (88.66%) with 80.33% of bud sprouting (Table-1). Contamination were also observed even after 3rd subculture because presence of endophytic contaminants. When lower concentration of sterilants employed, contaminants (bacterial and fungal) appeared on upper lower cut ends and near the buds. Chen and Yeh (2007) [9] also studied effect of antibiotic streptomycin for pretreatment of explants in aglaonema.

Effect of nodal position and size of explants

Explants from the 1st to 7th node positions were used for culture establishment. Explants from the 2nd node position survived the treatments, showed no contamination but also showed a poor response which is not suitable for *in vitro* propagation. The 3rd node position survived the treatment and showed a responsive outcome with less contamination (Table-2). They are considered suitable for culture establishment and *in vitro* propagation. Explants from the 4th node position survived the treatment but showed a high degree of contamination, hindering bud sprouting.

Effects of PGR on shoot initiation

During initiation of cultures, treatments exhibit that both applied growth regulators (BAP and Kin) and their concentration exerted significant effects on the initiation stage characters of single node explants of *A.* Red valentine grown *in vitro*. Treatments with higher concentrations of cytokinin, particularly in the range of 3.0 to 4.0 mg/l, tend to result in higher bud sprouting. The minimum 6 days are required for bud swelling and initiate bud sprouting. MS medium supplemented with 3.0 mg/l BAP and 4 mg/l KIN results in 84% and 78.66% of bud sprouting, respectively (Table-3).

Effects of PGR on multiplication of *A.* Red valentine

Treatments with varying concentrations of cytokinin (both BAP and KIN) influence the multiplication rate and average shoot height of *Aglaonema* cultures. Higher concentrations

of cytokinin generally results in increased shoot multiplication rates up to a certain point, after which the multiplication rate declines. The average shoot height tends to decrease as the concentration of cytokinin increases, indicating that higher concentrations may negatively affect shoot elongation. Shoots produced under all treatments exhibit overall healthy characteristics, including light green coloration and developed structures. No pigmentation seen in newly developed leaves and shoots. Pigmentation on leaves seen after 8 to 9 days of leaf emergence. Treatments with concentrations of 4 mg/l BAP resulted in healthy light green well developed shoots with 2.23 multiplication rate (Table-4). The control-basal MS medium (free of cytokinins) no multiplication rate of shoots (0.967) is observed. The data suggest that combinations of cytokinins (BAP and KIN) influence the shoot multiplication rate and average shoot height of *A.* Red valentine. But due to lack of PGR in medium shoot height is recorded maximum (9.33 cm). Medium supplemented with combinations of BAP and KIN resulted in varying multiplication rates and average shoot heights of cultures. Treatment 3, comprises 5 mg/l of BAP and 2 mg/l of Kinetin shows highest multiplication rate (2.53) among the treatments, but had a lower average shoot height (3.1 cm) (Table-5). This treatment exhibited a balance between multiplication rate and average shoot height of *A.* Red valentine.

In vitro rooting of *A.* red valentine

Analysis of the data provided in the table-6, which observes the effect of auxin on the rooting of *Aglaonema*. Treatments with combinations of IBA and NAA exhibited varying effects on rooting parameters. Generally, an increase in the concentration of IBA at certain level led to an increase in the percentage of rooting, and beyond this concentration rooting percentage declines with root number per shoot. Rooting of shoots observed in all the treatments tried during experiment. Treatment 16 (IBA 2.0 mg/l, NAA 0.5 mg/l) had the highest rooting percentage (95%) among the treatments with more roots per shoot (Table-6).

Hardening

The dataset outlines various combinations of potting mediums and their corresponding survival rates. These combinations include the use of Sand, Cocopeat, cocopeat: Sand, Cocopeat: Red soil and Cocopeat: red soil: FYM. The survival of plantlets is improved by covering them with polythene, which raises the relative humidity. From the data, it is evident that the cocopeat alone serves 90.83% survival of plantlets with 6.46cm of height days after plantation (Table-7). In contrast, Barakat and Gaber (2018) [10], uses mixture of perlite and peatmoss at (1:1); which resulted in the highest mean value of survival percentage/plant (100%).

Discussion

Similar to other ornamental aroids, fungal and bacterial contaminants being the major hurdle of *Aglonema* [11; 12]. Contamination were also observed even after 3rd subculture because presence of endophytic contaminants. When lower concentration of sterilants employed, contaminants (bacterial and fungal) appeared on upper-lower cut ends and near the buds. Use of antibiotic and fungicide in pre-sterilization technique is more efficient for *in vitro* asceptic culture initiation. Use of carbendazim and plantomycin (fungicide and antimicrobial agents, respectively) for reducing microbial load of explants. In present study, pre-treatment with carbendazim 0.1% and plantomycin (200 mg/l) caused lower contamination percentage. Similarly, Chen and Yeh (2007) [9] also reported that pre-treated with antibiotic mixtures containing streptomycin before *in vitro* culture could achieve satisfactory disinfection. As the concentration of $HgCl_2$ increased an increase in aseptic culture observed. Also it was observed that beyond certain limit of concentration and duration, exposure of explants to $HgCl_2$ causes phytotoxic effect.

Position of nodal segments plays an important role during establishment of cultures. Old buds contain more endophytic contaminants and immature buds do not survive after surface sterilization. To date, experiments on- effect of nodal position with respect to bud sprouting and asceptic culture establishment was not carried out in *Aglaonema*. Explants from the 3rd node position resulted more survival to the treatments with highest asceptic per cent. They are considered suitable for culture establishment and *in vitro* propagation of *Aglaonema*.

Endogenous level and exogenously supplied plant growth regulator plays an important role during regeneration of plantlets and these are species specific. To determine the optimum concentration and selective cytokinin for bud sprouting in *Aglaonema*, BAP and Kin were used at various concentrations in medium. During initiation of cultures, treatments exhibit that both applied growth regulators (BAP and Kin) and their concentration exerted significant effects on the initiation stage characters of single node explants. The minimum 6 days are required for bud swelling and initiate bud sprouting. MS medium supplemented with 3.0 mg/l BAP results in 84% of bud sprouting. However, study conducted by Barakat and Gaber (2018) [10] on *A. commutatum* shows the highest level of BA (1.00 mg/l); gave rise to the highest mean value of number of shoots per propagule (1.86). The data suggest that the BAP cytokinin perform best over the cytokinin- Kinetin. Higher concentrations of cytokinin generally results in increased shoot multiplication rates up to a certain point, after which the multiplication rate declines. In present study, it was revealed that MS medium fortified with concentrations of 4 mg/l of BAP produces healthy green well developed shoots with 2.23 multiplication rate. The average shoot height tends to decrease as the concentration of cytokinin increases, indicating that higher concentrations may negatively affect shoot elongation. Pigmentation on leaves was observed after 8 to 9 days of leaf emergence. Study of Zahara and Win (2020) [7] shows the best growth of axillary buds obtained on medium supplemented with 10 mg/l of BA. Fang *et. al.* (2013) [13], resulted TDZ as a best growth regulator for multiple shoot induction. Also, Gadhe and Kale (2020) [14], reported 4.0 mg/l BA + 1.0 mg/l NAA was found to be most suitable for shoot multiplication. Our results suggested that, MS medium supplemented with both cytokinin- BAP (5 mg/l) and Kinetin (2 mg/l) shows highest multiplication rate. Labasano (2018) [15] studied combination of Kin and NAA for the growth and development of *Aglaonema* tricolor cultures. The superiority of BAP over other cytokinins such as KIN, 2-iP and TDZ has been reported in *Aglaonema* sp. and some other ornamental Araceae [16, 17, 18, 19, 20].

Various researchers described IBA as a suitable auxin for root induction and also found to be superior to NAA and IAA [13; 21]. In present study, rooting of shoots observed in all the treatments tried. It was also observed that the

endogenous level of auxins in *Aglaonema* Red Valentine shoots enough for self inducing roots. IBA (2.0 mg/l) in combination with NAA (0.5 mg/l) shows the highest rooting percentage (95%) among the treatments tried. Similar, findings of Hussein (2001) [22] revealed that IBA at concentration of 2 mg/l in medium shows higher rooting.

Table 1: Effect of surface sterilization on explants.

Treatments	Sterilants	Conc. (%)	Time (min)	% Bud sprouting	% Aseptic culture	Major occurrence of contaminant
T_1	Ethanol	70	30 sec	0 (0)	0 (0)	Fungal and bacterial
	NaOCl	10	10			
T_2	Ethanol	70	30 sec	0 (0)	12.66 (20.8)	Fungal and bacterial
	NaOCl	10	15			
T_3	Ethanol	70	30 sec	0 (0)	16.33 (23.81)	Fungal and bacterial
	NaOCl	10	15			
	$HgCl_2$	0.1	5			
T_4	Ethanol	70	30 sec	54.00 (47.27)	24.00 (29.30)	Fungal and bacterial
	NaOCl	10	15			
	$HgCl_2$	0.1	10			
T_5	Ethanol	70	30	55.66 (48.23)	28.00 (31.93)	Fungal and bacterial
	NaOCl	10	15			
	$HgCl_2$	0.2	5			
T_6	Ethanol	70	1	74.33 (59.58)	57.00 (49.00)	Fungal and bacterial
	NaOCl	10	15			
	$HgCl_2$	0.2	10			
T_7	Ethanol	70	1	75.00 (59.98)	60.00 (50.74)	Fungal and bacterial
	NaOCl	10	15			
	$HgCl_2$	0.3	5			
T_8	Ethanol	70	1	74.66 (59.75)	64.33 (53.31)	Bacterial
	NaOCl	10	15			
	$HgCl_2$	0.3	10			
T_9	Ethanol	70	1	79 (62.70)	71.66 (57.82)	Bacterial
	NaOCl	10	15			
	$HgCl_2$	0.3	15			
T_{10}	carbendazim (0.2%) + Plantomycin (200 mg/l)		30	80.33 (63.65)	88.66 (70.32)	**Bacterial**
	Ethanol	70	1			
	NaOCl	10	15			
	$HgCl_2$	0.3	15			
C.D. $_{0.01}$				2.44 (1.6)	2.63 (1.88)	
SE (m)				0.82 (0.53)	0.88 (0.63)	

Table 2- Effect of nodal position and size of explants on initiation of *A.* Red valentine.

Node position from apical section	Length (cm)	Girth (cm)		Response of explants	Contamination (%)
	2	0.6	0.8		
1st				Not survive to treatments	No
2nd	✓	✓		Survive but shows poor response	No
3rd	✓	✓	✓	Survive to treatment, explants responsive with less contamination. Useful for *in vitro* propagation.	**Less**
4th				Survive to treatment, shows high degree of contamination. Hinders bud sprouting. Not suitable for *in vitro* propagation.	Maximum
5th					
6th					
7th					

= highest no. of average bud break & least occurrence of contamination.

Table 3: Effects of PGR on shoot initiation

Treatment	MS medium + Cytokinin		Bud sprouting (%)	Avg. no. of shoots produced per node	Avg. length of shoot per node (cm)	Minimum days require for bud swelling and sprouting
	BAP mg/l	KIN mg/l				
1	0.0	0	00.00 (00.00)	0	04.00	More than 15
2	0.5	0	11.00 (19.35)	1	04.00	9
3	1.0	0	15.66 (23.30)	1	09.00	8
4	1.5	0	28.00 (31.93)	1	09.00	7
5	2.0	0	32.66 (34.80)	1	12.00	7
6	2.5	0	53.33 (46.89)	1	12.33	6
7	3.0	0	84.00 (66.40)	1	15.00	**6**
8	3.5	0	75.00 (59.97)	1	11.33	6
9	4.0	0	08.33 (16.74)	1	02.00	6
10	4.5	0	13.00 (21.11)	1	03.00	6
11	5.0	0	18.00 (25.08)	1	05.00	7
12	0	0.5	27.00 (31.29)	1	07.00	13
13	0	1.0	33.66 (35.45)	1	06.66	12
14	0	1.5	38.00 (38.04)	1	07.00	10
15	0	2.0	47.00 (43.26)	1	08.33	9
16	0	2.5	58.66 (49.97)	1	09.00	8
17	0	3.0	64.33 (53.30)	1	11.00	6
18	0	3.5	68.00 (55.52)	1	11.00	6
19	0	4.0	78.66 (62.46)	1	12.00	6
20	0	4.5	65.33 (53.90)	1	09.66	7
21	0	5.0	60.33 (50.94)	1	08.66	8
$CD_{0.01}$			1.50(1.07)		2.06	
SE(m)			0.52(0.37)		0.72	

Table 4: Effect of PGR on multiplication of *A.* Red valentine

Treatment	MS medium + Cytokinin (mg/l)	Multiplication Rate	Avg. shoot height (cm)	Quality of shoots
1	**MS basal**	1: 1	8.66	Healthy light green, Developed shoots.
2	MS medium + 2 mg/l BAP	1: 1.3	8	Healthy light green, Developed shoots.
3	MS medium + 3 mg/l BAP	1: 1.6	6.33	Healthy light green, Developed shoots.
4	MS medium + 4 mg/l BAP	1: 2.23	6.66	Healthy light green, well Developed shoots.
5	MS medium + 5 mg/l BAP	1: 2.9	3.66	Healthy green, elongated well developed shoots.
6	MS medium + 6 mg/l BAP	1: 2.16	1.33	Healthy green, elongated well developed shoots.
7	MS medium + 1 mg/l KIN	1: 1	9.33	Healthy light green, Developed shoots.
8	MS medium + 2 mg/l KIN	1: 1	8	Healthy light green Developed shoots.
9	MS medium + 3 mg/l KIN	1: 1.4	7.66	Healthy light green, Developed shoots.
10	MS medium + 4 mg/l KIN	1: 1.9	4.33	Healthy light green, Developed shoots.
11	MS medium + 5 mg/l KIN	1: 1.96	4	Healthy light green, Developed shoots.
12	MS medium + 6 mg/l KIN	1: 2.03	2.83	Healthy light green, Developed shoots.
13	MS medium + 7 mg/l KIN	1: 2.5	2.7	Healthy green, elongated well developed shoots.
	$CD_{0.01}$	**0.22**	**2.18**	
	SE(m)	0.07	0.74	

Table 5: Effect of combination of cytokinin on shoot multiplication of *Aglonema.*

Treatment	Medium composition		In MS Basal medium	
	MS medium + BAP (mg/l)	MS medium + KIN (mg/l)	Multiplication rate	Avg. shoot height (cm)
1- Control	0	0	0.96	9.33
2	4	2	2.43	8.66
3	5	2	3.6	3.93
4	6	2	2.53	3.1
5	4	3	2.26	3.33
6	5	3	2.16	3.39
7	6	3	1.8	3.4
CD 0.01			0.24	1.67
SE(m)			0.08	0.53

Table 6: Effects of PGR on Rooting of *A.* red valentine

Treatment	Medium composition		% Rooting	No. of roots /shoot	Avg. length of roots (cm)
	MS medium + IBA (mg/l)	MS medium + NAA(mg/l)			
T_1	0.00	0.00	22.00 (27.96)	2	13
T_2	1.0	0	33.00 (35.04)	2.66	13.66
T_3	1.5	0	59.33 (50.36)	2	14.33
T_4	2.0	0	87.66 (69.43)	4	11.66
T_5	2.5	0	84.33 (66.67)	5	7
T_6	0	1.0	22.00 (27.96)	1.66	11.33
T_7	0	1.5	31.00 (33.81)	3	14.66
T_8	0	2.0	52.33 (46.31)	3	14.33
T_9	0	2.5	77.00 (61.32)	2.66	16
T_{10}	1.0	0.5	35.00 (36.25)	2	15
T_{11}	1.0	1.0	61.00 (51.33)	2.66	14.66
T_{12}	1.0	1.5	63.00 (52.51)	3	14.33
T_{13}	1.5	0.5	76.00 (60.64)	5	9
T_{14}	1.5	1.0	80.33 (63.66)	4	8.33
T_{15}	1.5	1.5	83.00 (65.62)	5	6.33
T_{16}	2.0	0.5	95.00 (77.09)	6	8
T_{17}	2.0	1.0	83.33 (65.89)	3.33	9.33
T_{18}	2.0	1.5	81.33 (64.38)	3	12
$CD_{0.01}$			1.97 (1.44)	2.31	2.81
SE(m)			0.68 (0.50)	0.80	0.97

Table 7: Effect of potting mixture on plantlets survival.

Potting mixture	Survival percentage	Average no. of leaves	Average plant height (40DAP) (cm)
Sand	10.20 (18.61)	4.30	4.46
Cocopeat	90.83 (71.86)	6.43	6.46
Cocopeat : sand (1:1)	59.30 (49.38)	4.53	5.36
Cocopeat : red soil (1:1)	60.56 (55.30)	4.50	5.16
Cocopeat : red soil : FYM (1:1:1)	70.46 (61.02)	5.43	5.80
$CD_{0.05}$	2.15 (1.58)	0.53	0.78
SE	1.05 (64)	0.16	0.32

DAP= Days after planting.

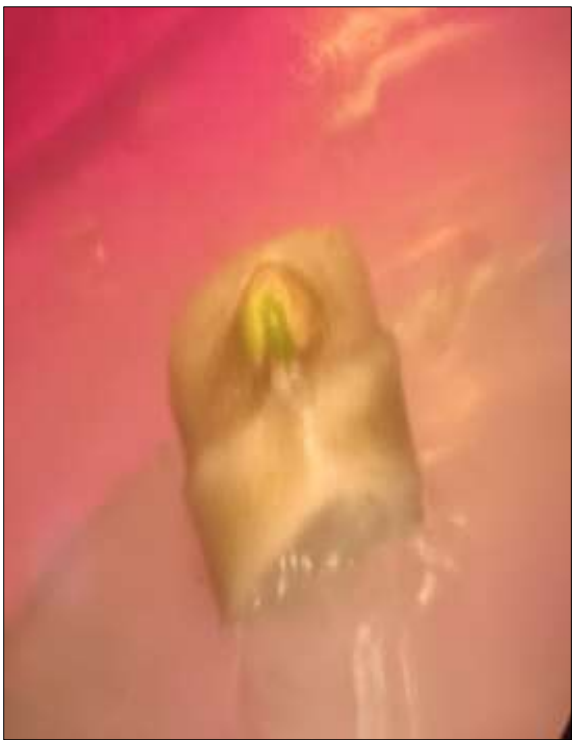

Fig 1: Establishment of Axillary bud.

Fig 2: Axillary bud sprouting

Fig 3: Multiplication of culture.

Fig 4: Development of shoots with pigmentation.

Fig 5: *In vitro* Rooting.

Conclusion

During culture establishment, use of pre-sterilization technique is helpful to reduce microbial load of explants. Our study revealed that explants treated with Carbendazim (0.2%) and Plantomycin (200 mg/l) for 30 minutes, increases percentage of asceptic cultures. Followed treatment with 70% Ethanol for 1 minute, 10% NaOCl for 15 minute and 0.3% $HgCl_2$ for 15 minutes, showed the highest aseptic culture percentages. Higher cytokinin concentrations (BAP and KIN) positively correlated with increased bud sprouting and shoot multiplication. Medium supplemented with two cytokinin, instead use of single, increases shoot multiplication rate. In our experiments use of both BAP (6 mg/) and KIN (2 mg/l) shows higher multiplication rate (2.53). Also, use of two auxins (IBA 2.0 mg/l, NAA 0.5 mg/l) in rooting medium shows highest rooting percentage (95%) among the treatments used. Use of Cocopeat (100%) in hardening of *Aglaonema* resulted 90% of survival of plantlets. This protocol provides a reliable and efficient method for the large-scale production of *Aglaonema* var. Red Valentine plants with uniform growth characteristics.

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