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In-vitro organogenesis studies in *Aglaonema var.* red valentine (*Aglaonema communtatum*), super white (*Aglaonema* spp.), red anjamani (*Aglaonema* spp.)

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Abstract
An efficient *in vitro* regeneration protocol was standardized for *Aglaonema* varieties Red valentine, Super white and Red anjamani using axillary bud explants. Sequential sterilization with Savlon, Tween-20, Carbendazim, Cefotaxime, ethanol, sodium hypochlorite and $HgCl_2$ achieved up to 88.66% aseptic cultures. Shoot initiation was optimal on MS medium with 3 mg/L BAP for Red valentine and 5 mg/L BAP for Super white and Red anjamani. Combined BAP + Kinetin gave maximum shoot multiplication (2.44 - 3.10 shoots/explant). Rooting was highest with IBA + NAA (94-96.43%). Cocopeat proved best for primary hardening and a 1:1 mix of red soil and cocopeat ensured >95% survival in secondary hardening. The protocol enables large-scale, disease-free, true-to-type Aglaonema production.

Keywords: Aglaonema, Red valentine, Super white, Red anjamani, Organogenesis, BAP, Kinetin, IBA, NAA

Introduction
Rising urbanization and reduced outdoor spaces have boosted demand for indoor foliage plants like *Aglaonema* (Chinese evergreen) valued for its attractive variegated leaves, shade tolerance, and air-purifying qualities. The global ornamental plant market, worth over USD 20 billion in 2024 continues to grow, with key Indian hubs in Bengaluru, Kolkata, Delhi, Pune and Thiruvananthapuram. Conventional propagation of *Aglaonema* is slow and limited while tissue culture offers rapid, large-scale, and disease-free production. However, optimizing sterilization, growth regulator balance and genotype responses remains challenging making protocol refinement vital for commercial production and germplasm conservation.

Materials
Genotypes
The study utilized three Aglaonema genotypes Red valentine (*A. commutatum*), Super white (*A. spp*.) and Red anjamani (*A. spp*.) selected for their ornamental value and high commercial demand in floriculture. Healthy, disease-free planting materials were obtained from nursery stock maintained at the College of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

Explant
Axillary buds were selected as explants due to their high regenerative potential and low contamination risk. Young shoots and nodal segments from field-grown plants were used as their meristematic tissues are ideal for *in vitro* organogenesis.

Culture medium
Murashige and Skoog (MS) medium was used for culture establishment enriched with

sucrose, agar, vitamins and amino acids. Various concentrations of cytokinins (BAP, Kinetin) were tested for shoot induction and multiplication and auxins (IBA, NAA) for rooting. The medium pH was adjusted to 5.8-5.9 and sterilized at 121 °C for 20 minutes.

Experimental Conditions

In vitro cultures were maintained aseptically at 25 ± 2 °C with a 16-hour photoperiod (1600 lux light intensity). Subculturing was done every 30-45 days to sustain proliferation and shoot vigor.

Methods

Preparation of Explant

Axillary buds were excised from mother plants washed with mild detergent and Tween-20 to remove debris, trimmed to uniform size and transferred to the tissue culture lab for sterilization under aseptic conditions.

Surface Sterilization

Explant sterilization involved sequential treatments with 70% ethanol, sodium hypochlorite (5-10%) and mercuric chloride (0.1-0.2%) to remove contaminants. Additional carbendazim (0.2%) and cefotaxime (250 mg/L) treatments controlled fungal and bacterial growth. Explants were rinsed thoroughly with sterile distilled water after each step.

Inoculation of Explant

Sterilized explants were inoculated on MS medium with varying BAP and Kinetin concentrations for shoot induction. Observations on bud break, response percentage and shoot number were recorded. Established shoots were sub cultured every 45 days to enhance multiplication.

Root Regeneration

Well-developed shoots were transferred to MS medium with different concentrations of IBA and NAA for root induction. Rooting was assessed based on rooting percentage, average roots per shoot and time for root initiation, ensuring development of complete plantlets for *ex vitro* transfer.

Statistical Analysis of Data

Experiments followed a Completely Randomized Design (CRD) with three replications of 20 explants each. Data on shoot initiation, multiplication, rooting and hardening were statistically analysed for reliability.

Result

Effect of Surface Sterilization

Sterilization treatments significantly affected contamination control and survival (Table 1). The best results were obtained with sequential treatment using 70% ethanol, sodium hypochlorite and mercuric chloride effectively reducing microbial load while maintaining explant viability.

Table 1: Effect of sterilization treatment on per cent aseptic culture and per cent survivability of *Aglaonema.*

Tr. No.	Sterilant	Conc.	Time	*Aglaonema var.*					
				Red valentine		Super white		Red anjamani	
				% Aseptic culture	% Survivability	% Aseptic culture	% Survivability	% Aseptic culture	% Survivability
T_0	Control (DDW washing)	-	30 min	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
T_1	Ethanol	70%	30 sec	5.72 (13.83)	4.84 (12.69)	6.11 (14.30)	4.00 (11.47)	6.32 (14.57)	5.20 (13.12)
	NaOCl	5%	2 min						
T_2	Ethanol	70%	60 sec	12.99 (21.11)	10.70 (19.08)	13.86 (21.83)	11.40 (19.71)	10.43 (18.83)	14.37 (22.24)
	NaOCl	5%	5 min						
T_3	Ethanol	70%	60 sec	16.33 (23.81)	24.27 (29.50)	18.66 (25.57)	28.17 (32.04)	17.66 (24.83)	26.23 (30.79)
	NaOCl	10%	5 min						
T_4	Ethanol	70%	60 sec	24.00 (29.31)	30.26 (33.35)	25.05 (30.02)	32.41 (34.68)	28.66 (32.35)	30.14 (33.28)
	NaOCl	10%	10 min						
T_5	Ethanol	70%	60 sec	36.38 (37.08)	43.54 (41.27)	36.52 (37.16)	42.29 (40.55)	35.66 (36.65)	42.30 (40.55)
	NaOCl	10%	20 min						
	HgCl2	0.1%	2 min						
T_6	Ethanol	70%	60 sec	58.33 (49.77)	45.66 (42.49)	43.29 (41.12)	46.40 (42.91)	46.66 (43.06)	48.36 (44.04)
	NaOCl	10%	10 min						
	HgCl2	0.1%	5 min						
T_7	Ethanol	70%	60 sec	61.00 (51.33)	52.47 (46.40)	48.10 (43.89)	52.12 (46.19)	54.33 (47.46)	52.66 (46.51)
	NaOCl	10%	10 min						
	HgCl2	0.1%	10 min						
T_8	Ethanol	70%	60 sec	64.33 (53.31)	60.17 (50.85)	51.97 (46.11)	58.32 (49.77)	58.33 (49.77)	59.03 (50.18)
	NaOCl	10%	10 min						
	HgCl2	0.15%	5 min						
T_9	Ethanol	70%	60 sec	71.66 (57.82)	75.56 (60.35)	68.21 (55.66)	71.00 (57.39)	67.33 (55.12)	72.53 (58.37)
	NaOCl	10%	10 min						
	HgCl2	0.15%	10 min						
T_{10}	Ethanol	70%	60 sec	83.83 (66.27)	82.20 (65.02)	75.02 (59.99)	84.61 (66.88)	72.66 (58.46)	80.26 (63.60)
	NaOCl	10%	10 min						
	HgCl2	0.20%	4 min						
T_{11}	Ethanol	70%	60 sec	88.66 (70.31)	90.08 (71.62)	85.00 (67.19)	92.14 (73.71)	78.66 (62.47)	89.17 (70.78)
	NaOCl	10%	10 min						
	HgCl2	0.20%	6 min						
SE(m)				0.68	0.44	0.44	0.51	0.53	0.56
CD 1%				2.73	1.78	1.77	2.04	2.13	2.23

In vitro Establishment of Axillary Shoot

Axillary buds showed good response on MS medium with cytokinins (Table 2). BAP alone at moderate concentrations gave the best bud break and establishment, ensuring healthy shoot development for further proliferation.

Table 2: Effect of plant growth regulators on shoot initiation of *Aglaonema*

Tr. No.	Media Combination	*Aglaonema var.* Red valentine % Bud sprouting			Super white % Bud sprouting			Red anjamn % Bud sprouting		
		% Bud sprouting	No. of shoot per node	Min. days require for sprouting	% Bud sprouting	No. of shoot per node	Min. days require for sprouting	% Bud sprouting	No. of shoot per node	Min. days require for sprouting
E1	MS (control)	0.00 (0)	0	-	0.00 (0)	0	-	0.00 (0)	0	-
E2	MS + 2.0 mg/l BAP	15.73 (23.35)	1	10	6.76 (15.06)	1	12	7.66 (16.06)	1	12
E3	MS + 2.5 mg/l BAP	47.10 (43.32)	1	7	18.20 (25.23)	1	11	11.12 (19.45)	1	11
E4	MS + 3.0 mg/l BAP	85.00 (67.19)	1	5	24.33 (29.54)	1	11	15.79 (23.40)	1	8
E5	MS + 3.5 mg/l BAP	74.80 (59.84)	1	6	36.08 (36.90)	1	9	27.18 (31.40)	1	8
E6	MS + 4.0 mg/l BAP	55.44 (48.10)	1	6	58.13 (49.66)	1	8	32.82 (34.93)	1	8
E7	MS + 4.5 mg/l BAP	46.54 (42.99)	1	7	63.66 (52.91)	1	8	53.46 (46.96)	1	7
E8	MS + 5.0 mg/l BAP	31.81 (34.32)	1	8	82.13 (64.97)	1	6	85.06 (67.25)	1	6
E9	MS + 5.5 mg/l BAP	23.64 (29.07)	1	10	75.53 (60.33)	1	6	63.46 (52.79)	1	7
E10	MS + 6.0 mg/l BAP	11.25 (19.57)	1	11	44.40 (41.76)	1	7	43.29 (41.12)	1	8
E11	MS + 6.5 mg/l BAP	7.07 (15.39)	1	11	12.00 (20.24)	1	10	17.36 (24.61)	1	12
E12	MS + 7.0 mg/l BAP	5.95 (14.11)	1	12	7.00 (15.31)	1	12	6.93 (15.26)	1	13
SE(m)		0.56			0.52			0.51		
CD 1%		2.26			2.07			2.05		

In vitro Shoot Regeneration

Shoot regeneration was mainly influenced by BAP concentration, with optimum levels promoting maximum response. Red valentine showed the highest regeneration and shoot number followed by Super white and Red anjamani indicating genotype dependent variation.

Subculturing for Multiple Shoot Regeneration

Subculturing established shoots in fresh multiplication medium significantly increased shoot production per explant (Table 3). The combined use of BAP and Kinetin proved most effective for multiple shoot formation. Regular subculturing further enhanced multiplication efficiency while maintaining shoot vigor and uniformity.

Table 3: Effect of combination of cytokinins on shoot multiplication of *Aglaonema.*

Tr. No.	Media combination	*Aglaonema var.* Red valentine		Super white		Red anjamani	
		Multi. rate	Avg. shoot height (cm)	Multi. rate	Avg. shoot height (cm)	Multi. rate	Avg. shoot height (cm)
SM1	MS + 4 mg/l BAP+ 1 mg/l Kin	2.44	3.00	2.40	4.00	2.62	4.02
SM2	MS + 4 mg/l BAP+ 2 mg/l Kin	2.23	2.80	2.60	4.00	2.44	4.00
SM3	MS + 5 mg/l BAP+ 1 mg/l Kin	2.10	2.80	2.63	4.82	2.66	4.32
SM4	MS + 5 mg/l BAP+ 2 mg/l Kin	2.10	4.00	2.80	5.33	2.32	4.60
SM5	MS + 6 mg/l BAP+ 1 mg/l Kin	1.80	4.20	2.30	4.50	3.10	5.20
SM6	MS + 6 mg/l BAP+ 2 mg/l Kin	1.71	4.00	2.10	4.20	2.80	5.00
SE(m)		0.05	0.12	0.09	0.29	0.13	0.19
CD 1%		0.22	0.53	0.41	1.26	0.58	0.86

In vitro Root Regeneration

Regenerated shoots on MS medium with auxins showed variable rooting responses (Table 4). IBA alone or combined with NAA was more effective than NAA alone for root induction. Lower auxin concentrations promoted faster rooting, whereas higher levels caused basal callus formation.

Table 4: Effects of PGR on Rooting of *Aglaonema*.

Tr. No.	Media Combination	*Aglaonema var.* Red valentine Rooting %	Red valentine Number of days for root induction	Super white Rooting %	Super white Number of days for root induction	Red anjamani Rooting %	Red anjamani Number of days for root induction
R1	MS (control)	12.00 (20.24)	40	10.35 (18.75)	41	9.00 (17.43)	40
R2	MS + 0.5 mg/l IBA	23.33 (28.87)	38	24.10 (29.38)	39	18.35 (25.34)	37
R3	MS + 1.0 mg/l IBA	32.21 (34.56)	38	34.33 (35.85)	39	36.66 (37.24)	37
R4	MS + 1.5 mg/l IBA	58.83 (50.06)	35	62.32 (52.11)	36	63.10 (52.57)	34
R5	MS + 2.0 mg/l IBA	87.66 (69.42)	31	89.05 (70.66)	32	86.41 (68.34)	31
R6	MS + 0.5 mg/l NAA	22.00 (27.95)	39	24.21 (29.45)	39	20.69 (27.04)	38
R7	MS + 1.0 mg/l NAA	30.70 (33.63)	37	31.38 (34.05)	37	28.86 (32.48)	36
R8	MS + 1.5 mg/l NAA	52.000 (46.12)	36	52.33 (46.31)	35	49.01 (44.41)	35
R9	MS + 2.0 mg/l NAA	77.02 (61.33)	35	77.63 (61.76)	34	78.45 (62.32)	34
R10	MS + 0.5 mg/l IBA + 0.5 mg/l NAA	37.27 (37.61)	37	36.33 (37.05)	38	34.65 (36.04)	36
R11	MS + 1 mg/l IBA+ 0.5 mg/l NAA	41.40 (40.03)	36	4 3.47 (41.23)	37	48.46 (44.10)	35
R12	MS + 1.5 mg/l IBA+ 0.5 mg/l NAA	76.15 (60.74)	30	78.34 (62.24)	33	74.63 (59.73)	29
R13	MS + 2 mg/l IBA + 0.5 mg/l NAA	95.33 (77.55)	28	52.34 (46.32)	31	86.52 (68.44)	29
R14	MS + 0.5 mg/l IBA + 1 mg/l NAA	44.66 (41.91)	37	44.74(41.96)	37	43.53 (41.26)	36
R15	MS + 1 mg/l IBA + 1 mg/l NAA	51.22 (45.68)	32	52 .45 (46.39)	35	52.33 (63.12)	34
R16	MS + 1.5 mg/l IBA+ 1 mg/l NAA	63.33 (52.71)	29	94.00 (75.82)	30	79.60 (49.77)	32
R17	MS + 2 mg/l IBA + 1 mg/l NAA	83.66 (66.13)	29	68.66(55.93)	29	96.43 (79.23)	28
SE(m)		0.55		0.58		0.60	
CD 1%		2.16		2.28		2.34	

Root Development

MS medium with 1.0-1.5 mg/L IBA produced well-developed roots with superior thickness, length and number per shoot, ensuring efficient root development and successful conversion of shoots into complete plantlets.

Hardening: Rooted plantlets were acclimatized in the greenhouse using various potting mixtures. The combination of red soil, cocopeat and vermicompost gave the highest survival (Table 5, Table 6) and growth. Hardening proved essential for successful transition from *in vitro* to natural conditions, ensuring plantlet survival and field establishment.

Table 5: Effect of potting mixtures on primary hardening on *Aglaonema*.

Tr. No.	Media Combination	*Aglaonema var.* Survival% Red valentine	Super white	Red anjamani
H1	Cocopeat	95.06 (77.18)	94.027 (75.82)	94.66(76.64)
H2	Red Soil + Cocopeat (1:3)	85.60 (67.67)	83.00 (65.62)	80 .00(63.41)
H3	Red Soil + Cocopeat (1:1)	65.56 (54.04)	67.00 (54.91)	67 .00 (54.92)
H4	Red Soil + Cocopeat + Vermicompost (4:5:1)	64.00 (53.11)	63.00 (52.51)	65.00 (53.70)
H5	Red Soil + Cocopeat + Vermicompost (1:2:1)	56.00 (48.42)	56.84 (48.91)	53.94 (47.24)
H6	Cocopeat + Vermicompost (3:1)	58.00 (49.584	60.00 (50.74)	62.00 (51.92)
H7	Red Soil + Cocopeat (3:1)	20.60 (26.97)	25.00 (29.98)	22.4 (28.23)
SE(m)		0.56	0.44	0.64
CD 1%		2.42	1.93	2.76

Table 6: Effect of potting mixtures on secondary hardening on *Aglaonema*. (45 days after)

Tr. No.	Media Combination	*Aglaonema var.* Survival% Red valentine	Super white	Red anjamani
H1	Cocopeat	90.82 (72.36)	91.00 (72.53)	89.00 (70.61)
H2	Red Soil + Cocopeat (1:3)	88.37 (70.0)	87.84 (69.57)	85.82 (67.86)
H3	Red Soil + Cocopeat (1:1)	95.14 (77.28)	96.15 (78.69)	97.00 (80.08)
H4	Red Soil + Cocopeat + Vermicompost (4:5:1)	89.61 (71.19)	87.59 (69.36)	90.16 (71.71)
H5	Red Soil + Cocopeat + Vermicompost (1:2:1)	77.07 (61.37)	74.96 (59.94)	72.22 (58.17)
H6	Cocopeat + Vermicompost (3:1)	73.73 (59.14)	64.85 (53.62)	68.29 (55.70)
H7	Red Soil + Cocopeat (3:1)	37.43 (37.74)	40.02 (39.22)	34.33 (35.85)
SE(m)		0.62	0.6	0.6
CD 1%		2.69	2.63	2.61

Discussion

Pre-treatment with antibiotic mixtures, particularly streptomycin, significantly improved disinfection efficiency in *Aglaonema* cultures (Chen and Yeh, 2007; Fang and Hsu, 2013) [4, 6], while sequential sterilization using $HgCl_2$, Tween-20, Savlon and Cefotaxime further enhanced aseptic culture establishment (Gehan, 2021; Sawardekar and Sherkar, 2024; Irawati *et al*., 2023) [8, 14, 9]. The addition of Tween-20 improved disinfectant penetration (Kaviani *et al*., 2019; Mariani *et al*., 2011) [10, 12] and Savlon increased sterilization efficiency (Abass *et al*., 2016) [1], confirming the effectiveness of integrated multi-step sterilization strategies. For shoot induction, 3.0 mg/L BAP resulted in the highest bud initiation in the Red valentine variety, consistent with previous findings (Ahamad, 2007; Kaviani *et al*., 2019) [2, 10]. Genotype-specific responses required optimized BAP concentrations, as higher levels reduced proliferation (Barakat and Gaber, 2018; El-Mahrouk *et al*., 2016; Mariani *et al*., 2011) [3, 5, 12]. The combination of moderate BAP and Kinetin enhanced shoot initiation and elongation, particularly in Super white, due to its higher meristematic sensitivity (Barakat and Gaber, 2018; Sawardekar and Sherkar, 2024) [3, 14]. Rooting was most successful with a combination of IBA and NAA, providing higher rooting percentages, better root morphology and optimal development (Sawardekar and Sherkar, 2024; Gehan, 2021; Abass *et al*., 2016; Labasano, 2018; Gadhe and Kale, 2023) [14, 8, 1, 11, 7]. While IBA promoted root initiation and NAA supported elongation, excessive auxin levels caused toxicity and reduced efficiency. Overall, these findings highlight that multi-component sterilization, genotype-specific cytokinin optimization and balanced auxin combinations are essential for efficient *in vitro* regeneration and rooting in *Aglaonema*.

Conclusion

An efficient *in vitro* regeneration protocol was developed for *Aglaonema* varieties Red valentine, Super white and Red anjamani using axillary bud explants. Sequential sterilization with Savlon, Tween-20, Carbendazim, Cefotaxime, ethanol, sodium hypochlorite and mercuric chloride achieved high aseptic culture success (78-89%). Optimal shoot establishment occurred on MS medium with 3-5 mg/L BAP, while shoot multiplication was maximized with BAP + Kinetin, producing 2.44-3.10 shoots per explant. Rooting was best with IBA + NAA (94-96%) and plantlet survival reached up to 97% after hardening in cocopeat and red soil mix. The standardized protocol enables large-scale production of true-to-type, disease-free *Aglaonema* plants, supporting commercial propagation and germplasm conservation.

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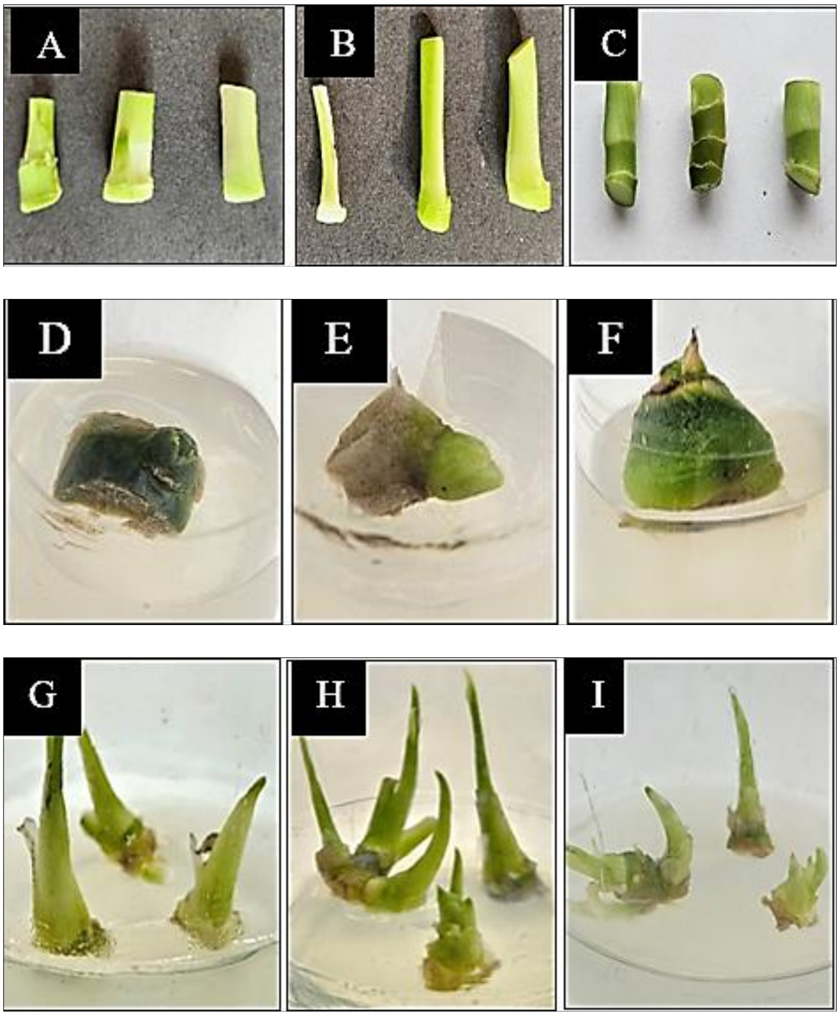

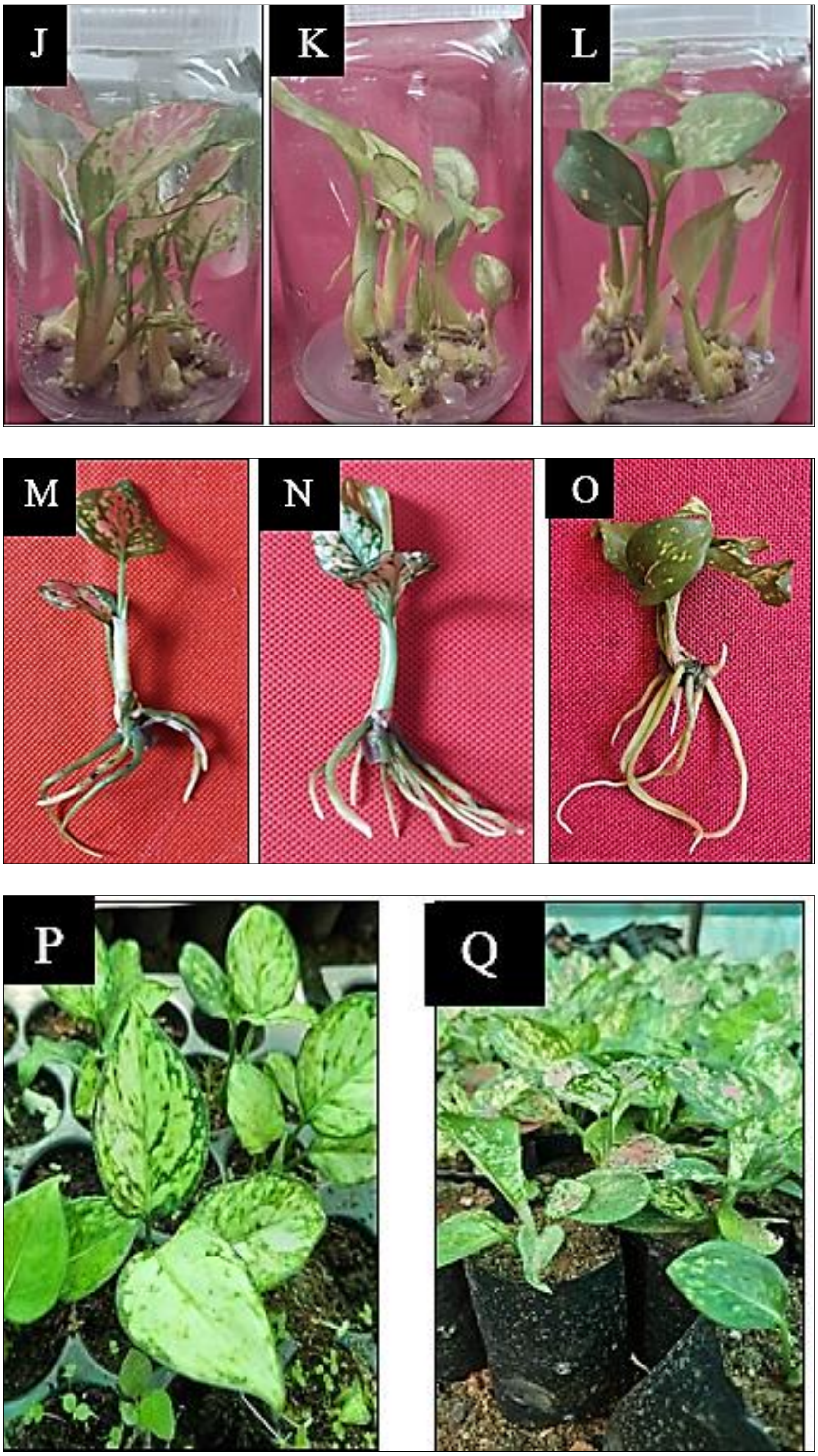

Fig 1: (A) Axillary Bud red valentine **(B)** Axillary Bud super white **(C)** Axillary Bud red anjamani **(D)** Bud sprouting in red valentine **(E)**Bud sprouting in super white **(F)** Bud sprouting in red anjamani **(G)** Shoot induction in red valentine **(H)** Shoot induction in super white **(I)** Shoot induction in red anjamani **(J)** Shoot multiplication in red valentine **(K)** Shoot multiplication in Super white **(L)** Shoot multiplication in red anjamani **(M)** Rooting in red valentine **(N)** Rooting in super white **(O)** Rooting in red anjamani **(P)** Primary hardening **(Q)** Secondary hardening

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