MICROPROPAGATION PROTOCOL OF RABBIT FOOT FERN *DAVALLIA FEJEENSIS* HOOK

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Abstract. This work was carried out in the Tissue Culture Laboratory, Horticulture Research Institute (HRI), Agricultural Research Center, Giza, Egypt during the years of 2018 and 2019 on the commercially important fern *Davallia fejeensis* Hook as an effort to establish a protocol to propagate this plant as much as possible in a relatively short time. For sterilization stage, the statistical analysis of data revealed that sterilization of explant with 0.1% mercuric chloride (MC) for 15 min is the suitable concentration and time for contamination-free and survival% explant. During the multiplication stage, the greatest leaf number, shootlet number and heaviest weight were observed in explants cultured in MS (Murashige and Skoog) medium supplemented with kinetin at 1.0 ppm. The highest value of total chlorophyll was achieved in the medium containing 0.5 ppm kinetin + NAA (1-Naphthaleneacetic acid**)** while carotenoid content rose to the highest content in medium free of plant growth regulators (control), 0.5 ppm BAP (6-Benzylaminopurine) or kinetin. For rooting stage, MS medium containing 4.0 ppm NAA produced the highest root number/plantlet, rhizome number and longest rhizome. Plants cultured in peat moss + sand + perlite produced the longest plant, greatest leaf number, fresh weight, greatest root number, longest root, greatest rhizome and longest rhizome number
Keywords: *MS-medium, ferns, mass multiplication, 6-benzylaminopurine, sterilization*

Introduction

Davallia fejeensis Hook commonly called rabbit foot fern belongs to the Davalliaceae family (Frohlich and Lau, 2008). It has furry rhizomes which cover the surface of the potting mixture as well as root down into it. The frond is triangular in shape and around 45 cm long and 30 cm wide. It divides into three to four pinnae which divide into many pinnules (Paul and Garber, 2015).

Ferns have been used extensively in the ornamental plant industry for landscaping and indoor. The beauty of their gently sloping leaflets makes an ideal accent in any setting. Most ferns like the shade, so it becomes an important accent to the darker portions of home and garden. Micropropagation of ferns *in vitro* has been tried using spores or vegetative parts. It can be applied especially for ferns that are hard to propagate conventionally for the benefit of the ornamental plant industry (Anico-Parr, 2000).

The ornamental plant is conventionally propagated by seeds and cutting, while propagation through seeds renders undesirable variation whereas shoot cuttings of many genotypes do not respond to root inducing medium (Lambardi and Rugini, 2003). In this regard, using micropropagation technique may be useful. Plant tissue culture is a tool for obtaining rapid, mass multiplication, diseases free and true to type plant material (Singh, 2003).

The first step in surface sterilization is the preparation of healthy explant for tissue culture because explants taken from field are highly exposed to microbial contamination

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(Sameer and Nabeel, 2016). Regardless of its serious health effect, mercury chloride is frequently utilized for surface sterilization for mitigating microbial contamination in sugarcane tissue culture (Tilahun et al., 2013).

Type and concentration of cytokinins were the most important factors affecting on shoot multiplication, (Romano et al., 2002). Cytokinins have important physiological effects for stimulating cell division as well as cell elongation by activating RNA synthesis and stimulating protein synthesis and enzyme activity (Kulaeva and Skoog, 1980). No clear stimulatory effect of zeatin riboside, and 2-iP effect on the nodes produced per explant of the fern *Marsilea quadrifolia* was reported when compared with hormone-free condition. On the contrary, the micropropagation of rhizome explant was inhibited, the inhibition decreased with the reducing strength of cytokinins (Rolli et al., 2015). The importance of auxin and its transport have been implicated in many physiological processes of roots, such as the regulation maintenance of root meristem and zonation (Luijten and Heidstra, 2009).

There is no micropropagation review on *Davallia fejeensis* Hook despite its aesthetic and coordination importance. Therefore, it was important to establish a protocol for micropropagation of *Davallia fejeensis* Hook.

Materials and methods

This work was carried out in the Tissue Culture Laboratory, Horticulture Research Institute (HRI), Agricultural Research Center; Giza, Egypt over two years from 2018 to 2019. This study was carried out to establish a protocol for micropropagation of *Davallia fejeensis* Hook.

Plant material

One specimen of the fern *Davallia fejeensis* Hook was obtained from the greenhouse of the Gene Bank, Genetic Engineering Institute, Agricultural Research Center (*Fig. 1*).

Figure 1. *Davallia fejeensis Hook*

Culture medium and incubation condition

The survived contamination-free explants were cultured in 250 ml/jar containing 25 ml of MS (Murashige and Skoog, 1962) basal medium supplemented with 30 g/l

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sucrose and solidified with 7 g/l agar. The medium pH was adjusted to 5.7 ± 0.1 with NaOH or HCl before sterilization by autoclaving at 121 °C for 20 min. The Plant growth regulators (PGR) were used according the experimental stage: benzylaminopurine (BAP), 6-Furfuryl-aminopurine (kinetin or kin), naphthalene acetic acid (NAA) or indole butyric acid (IBA). All cultures were incubated in room chamber at 24 ± 1 °C, under fluorescent illumination of 2000-2500 lux at 16\8 day\night fluctuation.

Sterilization stage

Pieces of the rhizomes were excised, washed with soap and water thoroughly for 15 min, rinsed under running water for 30 min. The explants were taken to aseptic conditions under the laminar air flow cabinet and soaked in either clorox at 5% (v/v) or mercuric chloride $HgCl_2$ (MC) at 0.1%, each for 5, 10, 15 or 20 min, with a few drops of Tween-20, followed by 3 rinses using distilled sterilized water. These pieces were divided to 1.5-2.0 cm long segments (explants) before being inoculated individually in MS medium free hormone for three weeks. Each treatment contained nine explants for three replicates. At the end of sterilization period contamination-free% and survived explants were calculated.

Multiplication stage

The survived contamination-free explants obtained from the new rhizomes were used in a multiplication experiment. These explants were inoculated on MS medium augmented with BAP or kin at (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 or 5.0 ppm), without or with NAA at (0.2 ppm). In each treatment nine explants with three replicates were cultured for three months (three subcultures). At the end of the 3rd subculture data were recorded] leaf number/cluster, shootlet number/explant, cluster height (cm), fresh weight (g), total chlorophyll and carotenoids (mg/g) fw)]. For pigments determination, the ethanolic extractions were submitted to procedures to determine the endogenous chlorophyll and carotenoids (mg/g fw) (Saric et al., 1967).

Rooting stage

Shootlets obtained from the new rhizomes developed *in vitro* in the previous multiplication experiment were used to investigate the effect of two auxins, namely NAA or IBA applied separately at 0.0, 1.0, 2.0, 3.0, 4.0 or 5.0 ppm on rooting of these shootlets for one month. Data were calculated at the end of this period [root number/plantlet, root length (cm), rhizome number/plantlet and rhizome length (cm)]. Each treatment contained nine shootlets in three replicates.

Acclimatization stage

Transplants (rooted plantlets) were transferred to plastic pots containing peat moss: sand: perlite (1:1:1 v/v/v), Peat moss: perlite (1:1 v/v) or perlite alone. These treatments were irrigated with solution of 0.1% rezolix fungicide and covered by transparent polyethylene bags. Each treatment contained nine plantlets in three replicates. The acclimatized transplants were out of door for two months. Plant height (cm), leaf number/plant, root number/plant, root length (cm), rhizome number/plant and rhizome length (cm) were measured.

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Statistical analysis

The design of experiments for starting, multiplication and rooting stage were factorial (two factor). While acclimatization experiment was one factor. The lay-out of the experiments was designed in complete randomized design and tests with program M-Stat. Least Significant Differences (L.S.D.) at $p \leq 0.05$ were used for the comparison means according to Steel and Torrie (1980).

Results and discussion

Sterilization stage

Effect of exposure to clorox and mercuric chloride (MC) for different times (min) on contamination-free% and survival% of Davallia fejeensis Hook

As shown in *Table 1*, there were significant fluctuations of exposure of clorox and mercuric chloride (MC) for different times and their interaction. For sterilization with clorox and MC, immersed explants in 0.1% MC recorded high percentage of contamination-free and survival % (47.25 and 66.75%, respectively) compared with using 5% (v/v) clorox for sterilization which gave 0.00% contamination- free and 52.75 survival%.

For different immersed times, explants immersed for 15 or 20 min gave high contamination-free % (27.83 or 39.00%) but reduced survival percentage to 44.33 or 38.67%. On the other hand, immersing explants for 5 or 10 min produced high % of survival (83.50 or 72.50%, respectively).

For the interaction between disinfection and times, 0.1% MC in all times gave suitable percentage of contamination-free% and survival %, while with 5% clorox all explants were contaminated in all times.

In this regard, Shekhawat and Manokari (2015) used mature rhizomes of *Marsilea quadrifolia* as explants and were successfully sterilized using 0.1% $HgCl_2$ for the establishment of cultures. Golamaully et al. (2015) reported that sterilization with 0.1% mercuric chloride resulted in minimal contamination levels but no germination of *Diplazium proliferum* spores were observed at all. Mercuric chloride at 0.05% was efficient for sterilization and the germination rate was 51%.

Table 1. *Effect of exposure in clorox and MC for different times on contamination-free% and survival% of Davallia fejeensis Hook*

Min	Contamination-free %			Survival %		
	Clorox (5%)	MC (0.1%)	Mean B	Clorox (5%)	MC (0.1%)	Mean B
5	0.00	11.00	5.50	78.00	89.00	83.50
10	0.00	44.33	22.17	67.00	78.00	72.50
15	0.00	55.67	27.83	33.00	55.67	44.33
20	0.00	78.00	39.00	33.00	44.33	38.67
Mean A	0.00	47.25		52.75	66.75	
$LSD_{0.05}$ A	11.96			13.52		
B	16.92			19.13		
A×B	23.93			27.05		

L.S.D. at 0.05 = Least Significant Different at 0.05 level of probability

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Multiplication stage

Effect of PGR concentration and type on leaf number/cluster and shootlet number/explant of Davallia fejeensis Hook

The illustrated data in *Table 2* showed a significant fluctuation in behavior of shooting (leaf number/cluster and shootlet number/explant). For types of PGR using kin either alone or with 0.2 NAA induced the greatest leaf and shootlet number (52.35 or 52.79 leaf/cluster and 10.33 or 10.75 shootlet/explant, respectively). However, the lowest record was a result of using BAP + NAA (28.12 leaf/cluster and 5.91 shootlet/explant, respectively).

For concentration of PGR, the greatest leaf number and shootlet number (48.83 leaf/cluster and 9.75 shootlet/explant) were produced when culturing explants on 1.0 ppm. The lowest number of leaves and shootlets resulted when using cytokinins at 4.0 or 5.0 ppm (36.50 or 35.75 leaf/cluster and 7.33 or 7.33 shootlet/explant, respectively).

For the interaction between PGR concentration and type, the greatest leaf and shootlet number in this concern belonged to explants treated with kin at 1.0 ppm (63.67 leaf/cluster and 12.67 shootlet/explant, respectively). The lowest record in the same regard was induced by the BAP + NAA at 4.0 or 5.0 ppm (19.33 or 14.67 leaf/cluster, and 4.00 or 3.66 shootlet/explant, respectively).

In this respect, Ravi et al. (2015) found that the highest number of *Pteris tripartita* sporophytes was induced by 4 mg/l BAP, while the lowest values were obtained by kinetin. Shekhawat and Manokari (2015) observed that maximum number of *Marsilea quadrifolia* shoots was achieved on MS medium augmented with 0.5 mg/l BAP.

Table 2. *Effect of PGR concentration and type on leaf number/cluster and shootlet number/explant of Davallia fejeensis Hook*

	Leaf number/cluster					**Shootlet number/explant**				
Conc. (ppm)	**BAP**	**BAP + NAA**	**Kin**	**Kin + NAA**	**Mean B**	**BAP**	**BAP + NAA**	**Kin**	**Kin + NAA**	**Mean B**
0.0	45.00	45.17	41.33	41.17	43.17	9.33	9.67	9.00	9.00	9.280
0.5	45.00	39.00	48.00	46.67	44.67	10.00	8.67	9.67	9.00	9.333
1.0	45.00	31.33	63.67	55.3	48.83	9.33	6.00	12.67	11.00	9.750
2.0	33.33	27.00	56.67	61.00	44.50	6.67	5.00	10.00	12.00	8.417
3.0	28.67	20.33	51.67	59.00	39.92	6.00	4.33	1.33	11.67	8.083
4.0	21.67	19.33	54.00	51.00	36.50	4.67	4.00	10.33	10.33	7.333
5.0	21.67	14.67	51.33	55.33	35.75	4.33	3.67	10.33	11.00	7.333
Mean A	34.33	28.12	52.38	52.78		7.190	5.905	10.33	10.57	
$LSD_{0.05}$ A			2.323					0.5627		
B			3.073					0.2625		
A×B			6.146					1.489		

L.S.D. at 0.05 = Least Significant Different at 0.05 level of probability

Effect of PGR concentration and type on cluster height (cm) and cluster fresh weight (g) on Davallia fejeensis Hook

The results shown in *Table 3* demonstrated that high significant differences were obtained for PGR type, concentration and their interaction on cluster height and cluster

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fresh weight. For PGR type, using kin or kin + NAA gave the tallest cluster and largest cluster fresh weight (2.512 or 2.567 cm and 2.307 or 2.492 g, respectively) compared to those produced in the presence of BAP or BAP + NAA (2.086 or 2.108 cm and 1.668 or 1.420 g, respectively).

PGR concentration affected cluster height and cluster fresh weight significantly, the tallest cluster and largest fresh weight (4.184 cm and 2.499 g, respectively) were noticed when no PGR were used (control). The shortest cluster and lowest fresh weight were a result of applying PGR at 5 ppm (1.772 cm and 1.635 g, respectively).

For the interaction between PGR concentration and type the tallest cluster resulted when explants were cultured on medium free hormone (control) (4.233, 4.167, 4.167 or 4.170 cm, respectively) while the heaviest fresh weight was produced when adding kin at 1.0 ppm or kin + NAA at 1.0, 2.0 or 3.0 ppm to culture medium which gave 2.65, 2.79, 2.76 or 2.76 g, respectively. On the other hand, the shortest cluster were observed when BAP at 5.0 ppm or BAP + NAA at 4.0 ppm were used which gave 1.58 or 1.45 cm, respectively while the lowest value of cluster fresh weight was recorded by BAP at 4.0 or 5.0 ppm or BAP + NAA at 4.0 or 5.0 ppm (0.92, 0.93, 0.94 or 0.85 g, respectively).

Findings of some workers were in harmony with our findings. Haddad and Bayerly (2014) mentioned that the longest stems of *Asplenium nidus* were observed when BAP 2.0 mg/l and IBA 0.3 mg/l were added. Ravi et al. (2015) found that the longest *Pteris tripartita* sporophytes were induced by 4 mg/L BAP, while the shortest ones were obtained by kin.

Table 3. *Effect of PGR concentration and type on plantlet height (cm) and plantlet fresh weight (g) on Davallia fejeensis Hook*

	Cluster height (cm)					**Cluster fresh weight (g)**				
Conc. (ppm)	**BAP**	**BAP + NAA**	**Kin**	**Kin + NAA**	**Mean B**	**BAP**	**BAP + NAA**	**Kin**	**Kin + NAA**	**Mean B**
0.0	4.23	4.17	4.17	4.17	4.184	2.74	2.74	2.21	2.30	2.499
0.5	1.63	2.07	2.92	3.27	2.473	2.02	1.69	1.90	1.97	1.896
1.0	1.83	1.78	2.58	2.57	2.192	2.03	1.36	2.65	2.79	2.207
2.0	1.67	1.72	2.05	2.45	1.971	1.54	1.24	2.30	2.76	1.960
3.0	1.60	1.68	1.93	1.95	1.791	1.51	1.11	2.54	2.76	1.978
4.0	2.05	1.45	2.25	1.63	1.847	0.92	0.94	2.20	2.44	1.627
5.0	1.58	1.88	1.69	1.93	1.772	0.93	0.85	2.34	2.43	1.635
Mean A	2.086	2.108	2.512	2.567		1.668	1.420	2.307	2.492	
$LSD_{0.05}$ A	0.1957					0.1907				
B	0.2588					0.2523				
A×B	0.5177					0.5046				

L.S.D. at 0.05 = Least Significant Different at 0.05 level of probability

Effect of PGR concentration and type on total chlorophyll and carotenoids content (mg/g fw) of Davallia fejeensis Hook

Data presented in *Table 4* showed the effect of PGR types, concentrations and their interaction. For the effect of PGR type, no significant differences in total chlorophyll were obtained by using different types of PGR while adding BAP raised the content of carotenoids compared to BAP + NAA, kin or kin + NAA.

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For concentration of PGR, the highest content of total chlorophyll was observed by adding 0.5 ppm (0.7930 mg/g fw) while the carotenoids raised in medium free hormone (control) or with 0.5 ppm which gave 0.3588 or 0.3327 mg/g fw, respectively.

For the effect of the interaction between PGR concentration and type achieved the highest position of total chlorophyll in medium containing 0.5 ppm kin + NAA while carotenes raised to the highest contents in medium free PGR (control), 0.5 ppm BAP or kin. The lowest contents of total chlorophyll were a result of applying kin at 2.0 ppm while carotenoids were the lowest when using kin at 5.0 ppm to MS medium.

In this respect, Gao *et a*l. (2000) on *Carthamus tinctorius* revealed that different concentrations of cytokinins and auxins had a significant effect on the chlorophylls and carotenoids formation capacity. In the same trend, Sayed and El-Kareim (2007) on *Cotoneaster horizontalis* studied the effect of different concentrations of cytokinins on the formation capacity of chlorophyll-a,-b and carotenoids. They observed that the production of such pigments was negatively related with the concentrations of the other cytokinins examined.

Table 4. *Effect of PGR concentration and type on total chlorophyll and carotenoids content (mg/g f.w.) of Davallia fejeensis Hook*

	Total chlorophyll content (mg/g fw)					**Carotenoids content (mg/g fw)**				
Conc. (ppm)	**BAP**	**BAP + NAA**	**Kin**	**Kin + NAA**	**Mean B**	**BAP**	**BAP + NAA**	**Kin**	**Kin + NAA**	**Mean B**
0.0	0.588	0.579	0.578	0.586	0.5826	0.360	0.359	0.359	0.357	0.3588
0.5	0.843	0.7413	0.684	0.904	0.7930	0.360	0.287	0.365	0.320	0.3327
1.0	0.524	0.411	0.318	0.475	0.4321	0.204	0.081	0.169	0.128	0.1455
2.0	0.349	0.397	0.187	0.237	0.2931	0.206	0.207	0.117	0.094	0.559
3.0	0.303	0.411	0.319	0.304	0.3343	0.233	0.104	0.097	0.061	0.1238
4.0	0.236	0.292	0.271	0.245	0.2610	0.146	0.135	0.030	0.090	0.1003
5.0	0.258	0.246	0.272	0.269	0.2611	0.157	0.203	0.014	0.088	0.1156
Mean A	0.4430	0.4396	0.3758	0.4315		0.2379	0.1967	0.1645	0.1625	
$LSD_{0.05}$ A	NS					0.04375				
B	0.08966					0.05788				
A×B	0.1793					0.1158				

L.S.D. at 0.05 = Least Significant Different at 0.05 level of probability (NS: non significant)

Rooting stage

Effect of auxin type and concentration on number of roots/plantlet and root length (cm) of Davallia fejeensis Hook

Data in *Table 5* show, significant differences between type, concentrations, and interaction of auxins with root number/plantlet and root length (cm). For type of auxins no significant differences were obtained for using NAA or IBA on root number/plantlet and root length (cm).

The effect of concentrations of auxins, MS medium containing 4.0 ppm produced the highest number of roots/plantlet (22.83 root/plant let) while the longest root length was recorded when using 0.0, 1.0, 3.0 or 4.0 ppm which gave 2.70, 2.642, 2.692 or 2.695 cm, respectively. On the other hand, using 5.0 ppm recorded the lowest root number and shortest root length (12.83 root/plantlet and 1.783 cm, respectively).

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For the interaction between concentrations and type of auxins, MS medium containing 4.0 ppm NAA or IBA produced the highest root number/plantlet (23.00 or 24.67 root/plantlet). In the same trend using 4.0 ppm IBA recorded the longest root length (3.40 cm). The lowest value for root number and root length was observed when using MS medium augmented with 5.0 ppm NAA which recorded 10.33 root/plantlet and 1.70 cm, respectively.

In this respect, Seyyedyousefi et al. (2013) on *Alstroemeria* (Alstroemerieae, ex. Liliaceae), Haddad and Bayerly (2014) on fern *Asplenium nidus*, Salem (2016) on *Aglaonema commutatum* and *Alocasia cucullata* and Badawy et al. (2020) on *Calathea medallion* reported that the highest number of roots and the longest roots were produced on medium with NAA.

Table 5. *Effect of auxin type and concentration on root number/plantlet and root length (cm) of Davallia fejeensis Hook*

	Root number/plantlet			**Root length (cm)**		
Conc. (ppm)	**NAA**	**IBA**	**Mean B**	**NAA**	**IBA**	**Mean B**
0	14.33	14.17	14.25	2.72	2.68	2.700
1	19.33	13.33	16.33	3.08	2.20	2.642
2	19.00	17.33	18.17	2.13	2.57	2.350
3	14.00	18.00	16.00	2.75	2.63	2.692
4	23.00	24.67	23.83	2.45	3.40	2.925
5	10.33	15.33	12.83	1.70	1.87	1.783
Mean A	16.67	17.14		2.472	2.558	
$LSD_{0.05}$ A		NS			NS	
B		1.832			0.8068	
A×B		2.590			1.141	

L.S.D. at 0.05 = Least Significant Different at 0.05 level of probability (NS: non significant)

Effect of auxin type and concentration on rhizome number (cm) and rhizom length (cm) of Davallia fejeensis Hook

Data presented in *Table 6* showed the effect of auxin type, concentration, and their interaction with number of rhizome/plantlet and rhizome length (cm). For auxin type, no significant differences were obtained between NAA or IBA on rhizome number/plantlet and rhizome length (cm).

The effect of auxin concentrations, medium augmented with 4.0 ppm has the highest rhizome number and longest rhizome length (9.00 rhizome/plantlet and 1.767 cm, respectively).

The interaction between auxin concentration and type, showed that the medium containing 4.0 ppm NAA recorded the highest number of rhizome and longest rhizome (10.00 rhizome/plantlet and 2.07 cm, respectively).

Acclimatization stage

Effect of media type on acclimatization for plant behaviour of Davallia fejeensis Hook

All rooted plantlets were transferred to different culture media of peat moss + sand + perlite, peat moss + perlite or perlite alone under ex vitro conditions. Data in *Table 7*

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indicated that, the media investigated gave significant differences in acclimatization on plant behavior. Cultured plants in peat moss + sand + perlite produced the longest plant, greatest leaf number, heaviest fresh weight, greatest root number, longest root, greatest rhizome number and longest rhizome (10.90 cm, 23.0 leaf/plant, 2.70 g 19.33 root/plant, 3.47 cm, 7.67 rhizome/plant and 3.33 cm, respectively). However, culturing in peat moss + perlite recorded the longest plant, root and rhizome (9.57, 4.68 and 3.73 cm, respectively). On the other hand, culturing plants in perlite depressed to minimum values recorded for growth of plant behavior. These results may be attributed to the high nutrition value of peat moss which can permit the *in vitro* plant to grow up in higher length comparing to the poor nutrition value of perlite or sand. In this regard, Sayed et al. (2005) on *Cereus peruvianus*, using peat moss alone or in combination with sand at 1:1 (v:v) allowed the stem of the *in vitro* acclimatized plants to grow up to the highest value.

Table 6. *Effect of auxin type and concentration on rhizome number/plantlet and rhizome length (cm) of Davallia fejeensis Hook*

	Rhizome number/plantlet			**Rhizome length (cm)**		
Conc. (ppm)	**NAA**	**IBA**	**Mean B**	**NAA**	**IBA**	**Mean B**
0	5.00	4.83	4.917	1.32	1.27	1.292
1	5.00	4.67	4.833	1.72	1.0	1.408
2	6.00	6.00	6.000	1.00	1.50	1.250
3	4.33	5.67	5.000	1.65	1.50	1.575
4	10.00	8.00	9.000	2.07	1.67	1.767
5	5.67	6.67	6.167	1.07	1.32	1.192
Mean A	6.000	5.972		1.496	1.358	
$LSD_{0.05}$ A		NS			NS	
B		1.662			0.4432	
A×B		2.351			0.6268	

L.S.D. at 0.05 = Least Significant Different at 0.05 level of probability (NS: non significant)

Table 7. *Effect of media type on acclimatization for plant behavior of Davallia fejeensis Hook*

	Plant height (cm)	**Leaf number**	**Fresh weight (g)**	**Root number**	**Root length (cm)**	**Rhizome number**	**Rhizome length (cm)**
Beat + sand + perlite	10.90	23.00	2.70	19.33	3.47	7.67	3.33
Beat + perlite	9.57	15.33	2.03	15.33	4.68	4.00	3.73
Perlite	6.57	17.33	1.35	6.00	1.65	2.33	1.28
$LSD_{0.05}$	1.628	5.902	0.7787	3.023	1.14	2.449	1.487

L.S.D. at 0.05 = Least Significant Different at 0.05 level of probability

Conclusion

Using 0.1% mercuric chloride (MC) for 15 min proved to be the suitable time and disinfection for contamination-free and survival% explant. For multiplication stage, adding 1.0 ppm kin to MS medium produce the greatest shooting behavior. During rooting stage, MS medium containing 4.0 ppm NAA showed the highest root

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number/plantlet, rhizome number and longest rhizome. Moreover, plants cultured in peat moss + sand + perlite produced good growth of plantlet (*Fig. 2*).

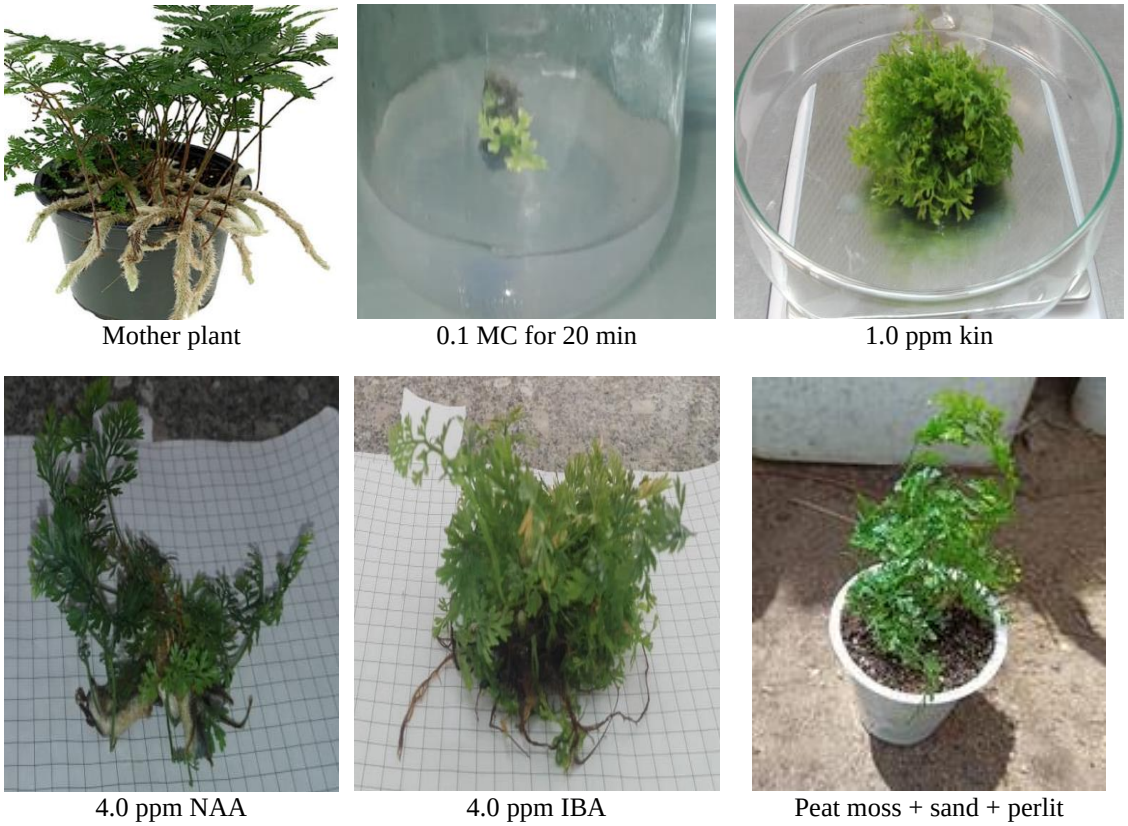

Figure 2. *Different stages on micropropagation of Davallia fejeensis Hook*

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