

PROTOCOL FOR IN VITRO MASS PRODUCTION OF *NEPHROLEPIS EXALTATA* SCHOTT (BOSTON FERN)

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Abstract. This study was carried out in the Tissue Culture Laboratory of Horticulture Research Institute (HRI), Agricultural Research Center, Giza, Egypt during the years of 2020 and 2021 on Boston fern (*Nephrolepis exaltata* Schott cv. *Bostoniensis*) to find a commercial method for in vitro mass production in the shortest time possible. The statistical analysis revealed that the ideal time for the sterilization of explants with 0.1% mercuric chloride (MC) was 15 min, which resulted in the highest survival rate and the lowest contamination percentage of explants (66.66 and 77.77%, respectively). For the multiplication stage, the highest shootlet number and leaf number were recorded for explants culture in MS medium supplemented with 1.0 mg/l BAP with 1 g/l AC (35.17 shootlet/explant and 5.17 leaf/shootlet). For rooting stage, full MS medium supplemented with 0.5 mg/l NAA and 1 g/l AC produced the highest rooting percentage, root number/plantlet, and longest root length (100.0%, 29.4 root/shootlet and 4.5 cm, respectively). Culturing plants in peat moss recorded the longest plant, also the greatest leaf number and the longest root (4.01 cm, 8.75 leaf/number and 1.97 cm, respectively).

Keywords: mercuric chloride, MS medium, cytokinins, auxins, activated charcoal

Introduction

Boston fern (*Nephrolepis exaltata* Schott cv. *Bostoniensis*) is an ornamental foliage plant considered today as one of the most marketable indoor pot plants (Hagiabad et al., 2007). *Nephrolepis exaltata* belongs to the Nephrolepidaceae family. It is native to North, Central, and South America (Karmakar et al., 2020).

Due to its improved ornamental value and higher tolerance to indoor environmental conditions, the mutant was named as *N. exaltata* 'Bostoniensis' and quickly gained its popularity as Boston fern (Schall et al., 2018).

Nephrolepis exaltata propagates usually asexually through long thin and green runners, (also through spores) and is a common houseplant commercially found in florist shops (Popovici, 2018). Due to the restriction of conventional propagation methods, mass propagation of this plant has increased through in vitro culture techniques (Hagiabad et al., 2007) and exploited as a common commercial method (Shafiei, 2008).

The efficient plant in vitro culture processes start with an optimal sterilization technique. The choice of time period and chemical agents depend on the sensitivity of the explant to be sterilized (Örge et al., 2018). There are a lot of common sterilants for the surface sterilization of plant material. Popular disinfectants are sodium hypochlorite, ethanol, mercuric chloride, calcium hypochlorite, silver nitrate, hydrogen peroxide, bromine water, and Tween 20 (Ishfaq, 2016).

Growth optimal of tissues may vary for different plants according to their nutritional requirements. Additionally, tissues from diverse parts of plants may have different

requirements for satisfactory growth (Sulaiman et al., 2020). Cytokinins and auxins are the most widely used plant growth regulators in in vitro plant culture and are usually used together. The ratio between cytokinins to auxins determining the type of culture was established or regenerated. In general auxins promote both cell division and cell growth while cytokinins promote cell division. A high auxin to cytokinin ratio generally favours root formation, while a high cytokinin to auxin ratio favours shoot formation. An intermediate ratio favours callus production (Sulaiman et al., 2020). Activated charcoal is commonly added to tissue culture media.

The effects of activated charcoal may be due to establishing a darkened environment; adsorption of undesirable/inhibitory substances; adsorption of growth regulators and other organic compounds, or the release of growth promoting substances present in or adsorbed by activated charcoal (Pan and Van Staden, 1998). Peat moss is one of the most important constituents of mixture media due to its capacity in affecting plant growth either indirectly or directly. Indirectly it improves the physical conditions of mixture media by enhancing aggregation, aeration (8%) and water retention (77%), thereby creating a suitable environment for root growth (Sensi and Loffredo, 1999). The aim of this research was to find commercial method for in vitro mass production for *Nephrolepis exaltata* Schott.

Materials and methods

This study was performed in the Tissue Culture Laboratory of Horticulture Research Institute (HRI), Agricultural Research Center; Giza, Egypt during the years 2020 and 2021 to find commercial method for in vitro mass production for *Nephrolepis exaltata* Schott.

Plant material

The plant sample of *Nephrolepis exaltata* Schott was obtained from the greenhouse of Al Zohriya Garden, Agricultural Research Center (*Fig. 1*).

Culture medium and incubation condition

The explants were cultured on 250 ml/jar containing 25 ml of MS (Murashige and Skoog, 1962) basal medium enriched with 25 g/l sucrose and solidified with 7 g/l agar. The pH medium 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 to the experimental stage: benzylaminopurine (BAP), 6-Furfuryl-aminopurine (kinetin or kin), isopentenyl adenine (2 ip) and naphthalene acetic acid (NAA). All cultures were stored in room chamber at 24 ± 1 °C, under fluorescent illumination of 2000-2500 lux at 16 \8 day\night fluctuation.

Sterilization stage

Runners of *N. exaltata* Schott were used as explants for in vitro cultures. Pieces of the runners were removed, thoroughly washed with soap and water for 15 min, rinsed under a running water for 1 h and then taken inside the laminar air flow cabinet for further sterilization. These segments were disinfected by soaking in either clorox at 5% (v/v) or mercuric chloride HgCl₂ (MC) at 0.1% each for (10, 15 or 20 min) with a few drops of Tween-20, then rinsed 3 times by a distilled sterilized water. These segments

were chopped to (1.0 cm) long segments (explants) before being put individually in - MS medium free hormones for three weeks. Each treatment included nine explants for three replicates. At the end of the sterilization period percentages of contamination-free, and surviving explants were calculated. Contamination-free and surviving explants were taken for multiplication stage.



Figure 1. *Nephrolepis exaltata* Schott (Boston fern)

Multiplication stage

The survived contamination- free explants were inoculated on MS medium fortified with BAP, kin or 2 ip at 0.0, 0.5, 1.0 or 1.5 ppm) with or without activated charcoal (AC) at (1 g/l). Each treatment was done in three replications with nine explants kept for two months (two subcultures). At the end of the second subculture data were noted [shootlet number/explant, leaf number/shootlet, shootlet length (cm), fresh weight (g), total chlorophyll and carotenoids (mg/g fw)]. For pigments determination, according to Saric et al. (1967) the ethanolic extractions were subjected to define the colour density to measure chlorophyll and carotenoids against the blank methanol.

Rooting stage

The multiplied shootlets were transferred to different MS strength (full, $\frac{3}{4}$, $\frac{1}{2}$ or $\frac{1}{4}$ strength) without or with NAA 0.5 ppm. All previous treatments with or without activated charcoal. Each treatment included nine shootlets in three replicates. After six weeks of culturing, rooting percentage, root number/plantlet, root length (cm) were recorded.

Acclimatization stage

After eight weeks, the rooted plantlets were carefully removed out of the jars and the roots were washed under running water. They were then cultured in plastic pots containing peat moss, peat moss: perlite (1:1 v/v), peat moss: perlite (2:1 v/v), peat moss: sand (2:1 v/v), or peat moss: sand: perlite (1:1:1 v/v/v). These pots were covered with polyethylene bags and maintained in a greenhouse for eight weeks to acclimatize. The polythene bags were progressively, taken away to expose the plantlets to the outer environment. Each treatment contained nine plantlet for three replicates. Data recorded at the end of acclimatization period were plantlet height (cm) leaf number/plantlet, root number/plantlet, root length (cm).

Statistical analysis

All experiments were factorial, except for the acclimatization experiment, which has one factor. The experiments were designed in complete randomized design. Least Significant Differences (L.S.D.) at $p \leq 0.05$ were used for the comparison of means according to Steel and Torrie (1980).

Results and discussion

Sterilization stage

Effects of various types, times and their interactions with sterilization agents on contamination-free% and survival% of Nephrolepis exaltata Schott.

The results on the effect of exposure to clorox and mercuric chloride (MC) for different times and their interaction are displayed in *Table 1*. The highest percentage of survival and contamination-free explants (70.37% and 81.48%, respectively) was obtained by treating the explants with 0.1% MC, while the reverse was observed with clorox, where it gave 59.25 survival% and 7.41% contamination- free explants.

Table 1. *Effect of various types, times and their interactions with sterilization agents on contamination- free % and survival % of Nephrolepis exaltata Schott.*

	Survival %			Free contamination %		
	5%Clorox	0.1%MC	Mean A	5%Clorox	0.1%MC	Mean A
10 min	77.77	88.89	83.33	0.00	66.66	33.33
15 min	55.55	66.66	61.11	11.11	77.77	44.44
20 min	44.44	55.55	50.00	11.11	100.0	55.56
Mean B	59.25	70.37		7.41	81.48	
LSD _{0.05}	A = 19.87 B = 24.34 A × B = 34.42			A = 11.07 B = 13.56 A × B = 19.18		

L.S.D. at 0.05 = least significant different at 0.05 level of probability

For the different immersing times, the maximum survival percentage (83.33%) was obtained in explants treated with sterilization agent for 10 min but this time decreased contamination- free explants to the lowest percentage (33.33%). The suitable time for survival and contamination free (61.11 and 44.44%) was recorded when immersing explants for 15 min in a disinfectant solution.

For the interaction between disinfections and time the results were as follows, exposure of explants for 10 min, in 0.1%. MC increased survival % to 88.88% and contamination- free explants to 66.66%, while using 5% clorox gave the lowest percentage of contamination- free in all cases.

Our results on sterilization of explants are in line with Ali et al. (2004) showing that 60-75% contamination free cultures of sugarcane were derived after treatment with 0.1 % HgC12 for 10 min. Yadav et al. (2017) on banana cv. Grand Naine, Guaranna et al. (2017) on *Punica granatum* reported that, the best results with lower contamination and higher explants survival % were recorded with 0.1% MC.

Multiplication stage

Effects of PGR, activated charcoal (AC) and their interactions on shootlet number/explant and shoot length (cm) of Nephrolepis exaltata Schott.

Data presented in Table 2 revealed the effects of PGR and activated charcoal (AC). For Shootlet number, the highest shootlet number was observed on MS medium supplemented with 1.0 mg/l BA, with an average number of 29.00 shoots per explants, On the other hand, kin at 1.0, 1.5 mg/l gave the lowest shootlet number (12.5 shootlets). Using AC increased shootlet number to 23.47 shootlet/explant compared to medium without AC which decreased shootlet number to 11.23 shootlet/explant. For the interaction between PGR and AC, the highest number of shootlets was a result of using 1.0 mg/l BAP with 1 g/l AC which recorded 35.67 shootlets, while applying of 1.5 mg/l kin without AC gave the lowest values (3.67 shootlets).

Table 2. *Effects of PGR, activated charcoal (AC) and their interactions on shootlet number/explant and Shoot length (cm) of Nephrolepis exaltata Schott.*

	Shootlet number/explant			Shoot length (cm)		
	Without AC	With AC	Mean A	Without AC	With AC	Mean A
Control	14.00	15.00	14.50	0.80	1.00	1.15
0.5 mg/l BAP	15.00	30.00	22.50	0.75	1.60	1.18
1.0 mg/l BAP	22.03	35.67	29.00	0.65	1.67	1.16
1.5 mg/l BAP	11.67	34.33	23.00	0.38	1.53	0.96
0.5 mg/l Kin	6.00	28.00	17.00	0.77	1.87	1.32
1.0 mg/l Kin	5.67	19.33	12.50	0.83	2.57	1.70
1.5 mg/l Kin	3.67	21.33	12.50	0.71	2.23	1.47
0.5 mg/l 2 iP	12.67	14.33	13.50	0.79	2.97	1.88
1.0 mg/l 2 iP	12.33	15.33	13.80	0.87	2.83	1.85
1.5 mg/l 2 iP	9.00	21.33	15.17	0.71	3.07	4.89
Mean B	11.23	23.47		0.726	2.183	
LSD _{0.05}	A = 3.454 B = 1.545 A×B = 4.885			A = 0.1811 B = 0.08098 A×B = 0.2561		

L.S.D. at 0.05 = least significant different at 0.05 level of probability

For shootlet length (cm), the longest shootlet was achieved for culturing explants on medium containing 2 iP at 0.5, 1.0 or 1.5 mg/l (1.88, 1.85, and 4.89 cm, respectively), while the shortest shootlets were recorded for culturing explants on MS medium free PGR (control) which gave 1.15 cm. For AC, MS medium supplemented with 1 g/l AC recorded the longest shootlet (2.183 cm), compared to the medium without AC which gave shortest shootlets (0.726 cm). For the interaction between PGR and AC, using 2 iP at 1.5 mg/l with AC gave rise to the longest shootlet (3.07 cm). The shortest shootlets were observed for BAP at 1.5 mg/l without AC (0.38 cm).

In this concern, Datta et al. (2006) on *Taxus wallichiana* and Stojicic et al. (2012) on *Pinus peuce* reported the lengthening effect of AC on internodes in plants. The use of activated charcoal in the intergeneric hybrid of orchid *Laeliocattleya amber* x *Brassocattleya pastoral* resulted in the greatest number of shoots (Villa et al., 2014). Souza et al. (2021) on *Cattleya crispata* revealed that adding activated charcoal after

ninety days of culture for in vitro multiplication of orchid *C. crispata* gave the longest and greatest number of shooting.

Effects of PGR, activated charcoal (AC) and their interactions on leaf number/shootlet and fresh weight (g) of Nephrolepis exaltata Schott.

Data presented in Table 3 showed significant differences between PGR, activated charcoal (AC) and their interactions. For leaf number, the effect of PGR, medium free PGR (control) resulted in the largest number of leaves (4.67 leaves), compared to other treatments. For AC, the medium containing AC recorded the greatest values of leaf number (4.063 leaf/shootlet) while the lowest values in the same interest were a result of using MS medium without AC (3.377 leaf/shootlet). For the interaction between PGR and AC, the greatest number of leaves was obtained by BAP at 1.0 mg/l with AC which produced (5.17 leaf/shootlet). The lowest values in the same concern were results of using BAP at 1.0 or 1.5 mg/l without AC (1.00 leaf/shootlet).

Table 3. *Effects of PGR, activated charcoal (AC) and their interactions on leaf number/shootlet and fresh weight (g) of Nephrolepis exaltata Schott.*

	Leaf number/shootlet			Fresh weight (g)		
	Without AC	With AC	Mean A	Without AC	With AC	Mean A
Control	4.90	4.43	4.67	0.70	1.80	1.25
0.5 mg/l BAP	1.17	5.03	3.10	0.82	2.43	1.63
1.0 mg/l BAP	1.00	5.17	3.08	0.79	1.93	1.36
1.5 mg/l BAP	1.00	4.63	2.82	0.52	2.10	1.31
0.5 mg/l Kin	4.33	4.27	4.30	0.84	2.57	1.71
1.0 mg/l Kin	4.23	3.87	4.05	0.83	1.60	1.22
1.5 mg/l Kin	3.93	4.33	4.13	0.77	1.83	1.30
0.5 mg/l 2 iP	4.87	2.97	3.92	1.53	1.57	1.55
1.0 mg/l 2 iP	4.27	2.73	3.50	1.40	1.27	1.33
1.5 mg/l 2 iP	4.07	3.20	3.63	1.03	1.90	1.47
Mean B	3.377	4.063		0.925	1.900	
LSD _{0.05}	A = 0.4262 B = 0.1906 A×B = 0.6028			A = 0.2123 B = 0.09495 A×B = 0.3003		

L.S.D. at 0.05 = least significant different at 0.05 level of probability

For fresh weight, the heaviest fresh weight of shootlet were obtained when 0.5 mg/l kin was used (1.71 g), while using kin at 1.0 mg/l or medium free PGR (control) gave the lightest fresh shootlet (1.22 and 1.25 g, respectively). For AC, MS medium supplemented with AC gave the heaviest fresh weight (1.90 g) than without AC (0.93 g). For the interaction, using 0.5 mg/l kin with AC resulted in the heaviest fresh weight (2.57 g). The lightest shootlets resulted when BAP without AC was applied which gave 0.52 g.

In this respect, Moraes et al. (2003) noticed that the best results for the plant fresh weight of Brazilian native orchids were observed with MS media supplemented with 1 g/l of activated charcoal (AC). Moreover, Lajayer et al. (2011) reported that the maximum shoot fresh weight was devoted to plantlets of potato grown on medium supplemented with 0.5% of AC in addition to the fresh weight of shoot increased with

ABA at 1 and 4 μ M. An et al. (2021) showed that the fresh weight of orchid species *Sedirea japonica* shootlet cultured in MS and 1/2 MS medium with activated charcoal and BAP was approximately 6 times higher than that of shootlet grown in MS and 1/2 MS medium without activated charcoal and BAP.

Effects of PGR, activated charcoal (AC) and their interactions on total chlorophyll (mg/g fw) and carotenoids (mg/g fw) of Nephrolepis exaltata Schott.

As shown in Table 4 there were significant correlations of PGR, activated charcoal (Ac) and their interactions. For total chlorophyll, the highest total chlorophyll content was a result of using kin at 1.0 mg/l, which recorded 1.59 mg/g fw, while the lowest one was obtained upon using BAP at 1.5 mg/l (0.88\ mg/g fw). For the effect of AC, medium including AC induced more total chlorophyll content (1.52 mg/g fw) compared to medium without AC which gave 1.03 mg/g fw. For the interaction, medium fortified with 1.0 mg/l kin with AC produced the highest content of total chlorophyll (1.95 mg/g fw) compared with using 1.5 mg/l BAP which recorded 0.47 mg/gfw.

Table 4. *Effects of PGR, activated charcoal (AC) and their interactions on total chlorophyll (mg/g fw) and carotenoids (mg/g fw) of Nephrolepis exaltata Schott.*

	Total chlorophyll (mg/g fw)			Carotenoids (mg/g fw)		
	Without AC	With AC	Mean A	Without AC	With AC	Mean A
Control	1.31	1.71	1.50	0.16	0.31	0.24
0.5 mg/l BAP	0.97	1.37	1.17	0.14	0.22	0.18
1.0 mg/l BAP	0.87	1.38	1.12	0.20	0.23	0.21
1.5 mg/l BAP	0.47	1.29	0.88	0.08	0.28	0.18
0.5 mg/l Kin	0.75	1.19	0.97	0.15	0.37	0.24
1.0 mg/l Kin	1.23	1.95	1.59	0.17	0.41	0.29
1.5 mg/l Kin	1.14	1.48	1.31	0.15	0.51	0.33
0.5 mg/l 2 iP	1.25	1.72	1.48	0.25	0.44	0.35
1.0 mg/l 2 iP	1.27	1.67	1.47	0.30	0.48	0.39
1.5 mg/l 2 iP	1.03	1.42	1.22	0.26	0.43	0.34
Mean B	1.03	1.52		0.19	0.36	
LSD _{0.05}	A = 0.3447 B = 0.1542 A×B = 0.4875			A = 0.08265 B = 0.03696 A×B = 0.1169		

L.S.D. at 0.05 = least significant different at 0.05 level of probability

Regarding carotenoids, applying 2 iP at 1.0 mg/l resulted in the highest content of carotenoids (0.39 mg/g fw) while the lowest carotenoids content was observed with BAP at 0.5 mg/l (0.18 mg/g fw). For AC, MS medium with AC produced more carotenoids content (0.36 mg/g fw) than without AC (0.19 mg/g fw). For the effect of the interaction between PGR and AC, kin at 1.5 mg/l with AC resulted in the highest carotenoids content (0.51 mg/g fw), while the lowest content (0.08 mg/g fw) was induced by BAP at 1.5 mg/l without AC.

These results coincided with the earlier data found by Tung Ming Sung (1997) on *Cucumis sativus* and Gao et al. (2000) on *Carthamus tinctorius*. They showed that the different concentrations of cytokinins had a significant effect on the chlorophylls and carotenoids formation capacity.

Rooting stage

Effects of MS strength medium, activated charcoal (AC) and their interactions on rooting percentage, root number/shootlet and root length (cm) of Nephrolepis exaltata Schott.

The illustrated values in Table 5 showed significant differences resulted for MS strength medium and AC and their interactions. For rooting percentage, the highest rooting percentage was achieved in $\frac{3}{4}$ MS with or without 0.5 mg/l NAA or full MS plus 0.5 NAA (94.44%), while the lowest values resulted from $\frac{1}{2}$ MS strength plus 0.5 NAA (72.22%). For the effect of AC, the highest rooting percentage was obtained from MS with AC (94.44%), while MS without AC the lowest rooting ratio (79.16%) was achieved. The interaction between MS strength and AC resulted in greater percentage of rooting in most of the treatments, with a percentage ranging between 100, 88.89 and 77.77% while the lowest rooting percentage (44.44%) resulted when $\frac{1}{2}$ MS plus 0.5 NAA without AC was used.

Table 5. *Effects of MS strength medium, activated charcoal (AC) and their interactions on rooting percentage, root number/shootlet and root length (cm) of Nephrolepis exaltata Schott.*

	Rooting%			Root number/shootlet			Root length (cm)		
	Without AC	With AC	Mean A	Without AC	With AC	Mean A	Without AC	With AC	Mean A
Full MS	77.77	100.0	88.89	10.54	23.20	16.87	0.65	1.13	0.89
$\frac{3}{4}$ MS	88.89	100.0	94.44	11.23	16.10	13.67	0.65	0.91	0.78
$\frac{1}{2}$ MS	77.77	77.77	77.77	3.78	3.77	3.78	0.36	0.27	0.32
$\frac{1}{4}$ MS	88.89	77.77	83.33	6.88	7.89	7.39	0.75	0.65	0.70
Full + 0.5NAA	88.89	100.0	94.44	11.53	29.40	20.47	0.59	4.50	1.05
$\frac{3}{4}$ + 0.5NAA	88.89	100.0	94.44	10.33	16.57	13.45	0.58	1.46	1.02
$\frac{1}{2}$ + 0.5NAA	44.44	100.0	72.22	2.44	7.44	4.94	0.28	0.76	0.52
$\frac{1}{4}$ + 0.5NAA	77.77	100.0	88.89	4.33	10.53	7.43	0.57	0.99	0.78
Mean B	79.16	94.44		7.634	14.36		0.554	0.958	
LSD _{0.05}	A = 20.77 B = 10.38 A×B = 29.37			A = 1.315 B = 0.658 A×B = 1.860			A = 0.348 B = 0.172 A×B = 0.486		

L.S.D. at 0.05 = least significant different at 0.05 level of probability

For root number, it was noticed that the highest root number recorded by using full MS plus 0.5 mg/l NAA which recorded 20.47 roots, while the lowest number of roots resulted from using $\frac{1}{2}$ MS without or with 0.5 mg/l NAA (3.78 or 4.94 roots, respectively). For AC, it was observed that MS with AC gave more roots (14.36 roots) than MS without AC (7.63 roots). For the interaction between MS strength medium and AC, applying full MS plus 0.5 NAA with AC gave rise to the highest number of roots (29.40 roots). The lowest value in the same concern was obtained when $\frac{1}{2}$ MS plus 0.5 NAA without AC was applied (2.44 roots).

For root length, it was observed that the longest root (1.05 cm) was observed by using full MS plus 0.5 mg/l NAA, while the shortest roots resulted when using $\frac{1}{2}$ MS without NAA (0.32 cm). In respect to AC, MS with AC gave longer roots (0.958 cm) than MS without AC (0.554 cm). For the interaction between MS strength and AC, the

longest root (4.50 cm) was recorded from cultured shoots on full MS plus 0.5 mg/l NAA, while, the shortest roots were a result from using ½ MS with AC (0.27 cm).

A wide survey of the obtained data indicated that the shoots of *Fortunella crassifolia* were obtained from epicotyl explants, that recorded maximum rooting (75%) on ½ MS medium supplemented with NAA, kin and AC (Yang et al., 2006). Haddad and Bayerly (2014) remarked that during rooting of the fern *Asplenium nidus*, the maximum root length was observed when NAA was added at 0.5 mg/l. Dev et al. (2015) found that supplementing with AC was important not only for promoting the rooting frequency, but also for improving overall root quality, essential for subsequent ex vitro survival. Seleem and Taha (2021) reported that half strength MS medium with 0.5 mg L-1 NAA gave the lowest values of rooted shoots percentage, number of roots/shoot, and average root length (cm) (75%, 4.0, 3.8 cm, respectively).

Acclimatization stage

Effects of various substrate types on acclimatization for plant behaviour of Nephrolepis exaltata Schott.

Data presented in Table 6 showed significant differences between the effects of various substrates on vegetative growth (plantlet length and leaf number/plantlet), while non significant differences on root growth (root length and root number/plantlet). The plantlets acclimatized in peat moss alone gave the longest plant and greatest leaf number (4.01 cm and 8.75 leaf/plant, respectively). On the other hand, acclimatized plants in Peat moss + Sand (1:1 v: v) produced the shortest plants (2.78 cm), while the lowest number of leaves was obtained from using peat moss + sand (2:1 v: v) which gave 6.17 leaf/plantlet.

Table 6. *Effects of various substrate types on acclimatization for plant behaviour of Nephrolepis exaltata Schott.*

	Plantlet length (cm)	Leaf number/plantlet	Root length (cm)	Root number/plantlet
Peat moss	4.01	8.75	1.97	6.33
Peat moss + Perlit (1:1)	3.00	7.70	1.43	7.67
Peat moss + Perlit (2:1)	2.93	8.55	1.33	7.67
Peat moss + Sand (1:1)	2.78	6.75	1.10	6.33
Peat moss + Sand (2:1)	3.15	6.17	1.70	6.67
Peat moss + Perlit + Sand (1:1:1)	3.71	7.75	1.27	7.67
LSD _{0.05}	1.214	1.581	NS	NS

L.S.D. at 0.05 = least significant different at 0.05 level of probability (NS: Non significant differences)

In this concern, Kurtar et al. (2010) transplanted plantlets of winter squash (*Cucurbita maxima*) to soil, sand, perlite and peat moss, to decide the growth media effects on growth and survival of in vitro plantlets during acclimatization stage. They found that the highest survival % and plant height were recorded from peat moss as a potting medium while the lowest values were resulted when sand was used, in the same request. Abbas et al. (2011) reported that the in vitro derived plantlets of *Zingiber officinale* were acclimatized in plastic trays including different soil mixture. They revealed that the highest survival% (100%) was observed when only peat moss was used. Other mixtures, i.e. peat moss: sand (1:1), peatmoss: sand: perlite (1:1:1) and peat

moss: sand: vermiculite (1:1:1) recorded 80, 80 and 60% survival percentage, respectively. Wang et al. (2013) remarked that survival ratio of *Cymbidium lowianum* plantlets (Orchidaceae) was up to 92 percent in peat moss after 1 month of acclimatization. Taha et al. (2018) acclimatized plantlets of *Dillenia indica* successfully in peat moss alone or a mixture of peat moss + perlite + sand.

Conclusion

Data in *Figure 2* revealed that, sterilization explant with 0.1% mercuric chloride (MC) for 15 min the suitable time and disinfection for survival and free contamination explant percentage. For multiplication stage, the greatest shootlet number and leaf number were recorded in explants culture in MS medium supplemented with 1.0 mg/l BAP with 1 g/l AC. On rooting stage, full MS medium supplemented with 0.5 mg/l NAA and 1.0 g/l AC produced the highest rooting percentage, root number/plantlet, and the longest root length. Culturing plantlet in peat moss recorded the longest plant, the greatest leaf number and the longest root.

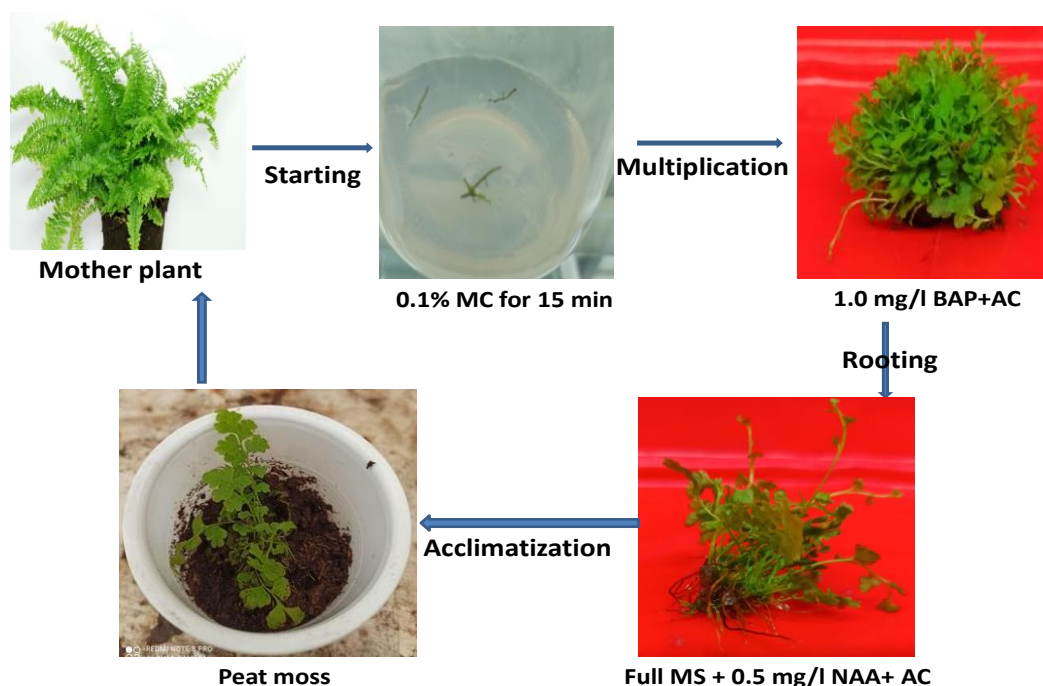


Figure 2. Different stages on micropropagation of *Nephrolepis exaltata* Schott.

REFERENCES

- [1] Abbas, M. S., Taha, H. S., Aly, U. I., El-Shabrawi, H. M., Gaber, E. S. I. (2011): In vitro propagation of ginger (*Zingiber officinale* Rosco). – Journal of Genetic Engineering and Biotechnology 9(2): 165-172.
- [2] Ali, S., Hassan, S. W., Razi-ud-Din, S., Shah, S., Zamir, R. (2004): Micropropagation of sugarcane through bud culture. – Sarhad J. Agric. 20(1): 79-82.

- [3] An, J., Kim, P. B., Park, H. B., Kim, S., Park, H. J., Lee, C. W., Lee, B. D., Kim, N. Y., Hwang, J. E. (2021): Effects of different growth media on in vitro seedling development of an endangered orchid species *Sedirea japonica*. – *Planta* 10(6): 1193.
- [4] Datta, M. M., Majumder, A., Jha, S. (2006): Organogenesis and plant regeneration in *Taxus wallichiana* (Zucc.). – *Plant Cell Reports* 25: 11-8.
- [5] Dev, R., Singh, S. K., Singh, A. K., Verma, M. K. (2015): Comparative in vitro multiplication of some grape (*Vitis vinifera*) genotypes. – *Ind. J. Agr. Sci.* 85: 1477-1483.
- [6] Dharishini, M. P., Moorthy, M. K., Balasubramanian, K. (2015): Effects of plant growth regulators and activated charcoal on regeneration and plantlet development in Neer Brahmi (*Bacopa monnieri*). – *J Acad Ind Res* 4: 69-74.
- [7] Gao, W. Y., Fan, L., Paek, K. Y. (2000): Yellow and red pigment production by cell cultures of *Carthamus tinctorius* in a bioreaction. – *Plant Cell, Tissue & Organ Culture* 60(2): 95-100.
- [8] Guaranna, P., Hosamani, I., Sathyanarayana, R., Hegdeand, R., Hipparagi, K. (2017): Micropropagation in pomegranate (*Punica granatum* L.) cv. 'Bhagwa' through Indirect Organogenesis and assessment of genetic fidelity by RAPD marker. – *Biotechnology Journal International* 20: 1-8.
- [9] Haddad, S., Bayerly, R. (2014): In vitro propagation of ferns (*Asplenium nidus*) via spores culture. – *Jordan J. Agric. Sci.* 10(1): 144-153.
- [10] Hagiabad, M. S., Hamidoghli, Y., Gazvini, R. F. (2007): Effects of different concentrations of mineral salt, sucrose and benzyladenine on Boston fern (*Nephrolepis exaltata* Schott cv. *Bostoniensis*) runner tips Initiation. – *JWSS-Isfahan University of Technology* 11(40): 137-146.
- [11] Ishfaq, S., Ahmed, S. D., Shah, H. A., Khan, R. T., Bukhari, S. M. F., Hameed, I., Mubeen, H., Awan, N., Abbas, S. R., Raza, S. (2016): In-vitro optimization protocol of wheat cultivars in newly established lab of plant culture, *Muzaffarad*. – *Euro J Pharma and Med Res* 3(3): 477-479.
- [12] Joshi, P., Dhawan, V. (2007): Assessment of genetic fidelity of micropropagated *Swertia chirayita* plantlets by ISSR marker assay. – *Biol. Plant.* 51: 22-26.
- [13] Karmakar, B., Chakrabarty, S., Hayat, A., Bagchi, S. (2020): Processing of *Nephrolepis exaltata* with glycerine to enhance shelf life by drying. – *Int. J. Curr. Microbiol. App. Sci.* 9(3): 348-356.
- [14] Kurtar, E. S., Balkaya, A., Özbek, N. (2010): Effects of polymers and growth media on in vitro plantlets of winter squash (*Cucurbita maxima* Duch. ex Lam.) and pumpkin (*Cucurbita moschata* Duch. ex Poir.) in acclimatization. – *Annals of Biological Research* 1(2): 148-154.
- [15] Lajayer, H. M., Esmailpour, B., Chamani, E. (2011): Hinokitiol and activated charcoal influence the microtuberization and growth of potato (*Solanum tuberosum* cv. *Agria*) plantlets in vitro. – *Australian Journal of Crop Science* 5(11): 1481-1485.
- [16] Moraes, L. M., Faria, R. T., Cuquel, F. L. (2003): Activated charcoal for in vitro propagation of Brazilian orchids. – *V International Symposium on New Floricultural Crops* 683: 383-390.
- [17] Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bio-assay with tobacco tissue culture. – *Physiolgia Plantarum* 15: 473-497.
- [18] Öргеç, M., Karakaş, F. P., Şahin, G., Ferdi, A. Ğ. I. L., Zencirci, N. (2018): Einkorn (*Triticum monococcum* ssp. *monococcum*) in vitro propagation sterilization protocol. – *Inter. J. of Secondary Metabolite* 5(2): 67-74.
- [19] Pan, M. J., Van Staden, J. (1998): The use of charcoal for in vitro culture. A review. – *Plant Growth Regulation* 26(3): 155-163.
- [20] Popovici, P. C., Ancuceanu, V. R., Oлару, T. O., Stoicescu, C. S., Dinu, M., Ancuceanu, V. R. (2018): Toxicity assessment of *Nephrolepis exaltata* (L.) Schott, Fam. Nephrolepidaceae. – *Acta Biologica Marisiensis* 1(1): 26-35.

- [21] Saric, M.; Kostrori, R.; Cupina, T., Geric, I. (1967): Chlorophyll determination. – Univ. Noven Sadu Praktikum is kiziologize Bilijaka Beogard, Haucana, Anjiga.
- [22] Schall, W., Huo, H., Chen, J. (2018): Cultural Guidelines for Commercial Production of Boston fern (*Nephrolepis exaltata* ‘Bostoniensis’). – EDIS 2018(1).
- [23] Seleem, E., Taha, Z. K. (2021): Effect of plant growth regulators on in vitro direct organogenesis of *Paulownia tomentosa* plant. – Scien. J. of Agric. Sci. 3(1): 111-118.
- [24] Sensi, N., Loffredo, E. (1999): The Chemistry of Soil Organic Matter. – In: Spark, D. L. (ed.) Soil Physical Chemistry. CRC Press, Boca Raton, FL, pp. 239-370.
- [25] Shafiei, H. M., Hamidoughli, Y., Fotouhi, G. R., Fatahi, M. J. (2008): The effects of different nutrient media on shoot proliferation of boston fern (*Nephrolepis exaltata* Schott cv. Bostoniensis). – Iranian Journal of Horticultural Science and Technology 2(9): 139-152.
- [26] Souza, D. M. S. C., Fernandes, S. B., Molinari, L. V., Avelar, M. L. M., Brondani, G. E. (2021): Activated charcoal application for the micropropagation of *Cattleya crispata* (Thunb.) – Van den Berg. Nativa 9(4): 352-358.
- [27] Steel, R. G. D., Torrie, J. H. (1980): Principles of Statistics. A Biometrical Approach. 2nd Ed. – Mc Graw-Hill Kogakusha, Kawasaki.
- [28] Stojicic, D., Janosevic, D., Uzelac, B., Cokesa, V., Budimir, S. (2012): Micropropagation of *Pinus peuce*. – Biologia Plantarum 56: 362-364.
- [29] Sulaiman, S., Yusuf, N. A., Awal, A. (2020): Effect of plant growth regulators on in vitro culture of pineapple (*Ananas comosus* L. Merr) MD2 variety. – Food Research 4(5): 110-114.
- [30] Taha, L. S., Sayed, S. S., Farahat, M. M., El-Sayed, I. M. (2018): In vitro culture and bulblets induction of Asiatic hybrid lily ‘Red Alert’. – J. Biol. Sci. 18: 84-91.
- [31] Tung Ming Sug, S. (1997): Cytokinins-efficiently enhanced pigment production in detached cotyledons of dark-grown cucumber seedlings. – Jour. of Agric. & Forest. 46(2): 85-91.
- [32] Villa, F., Pasqual, M., Silva, E. F. (2014): Micropropagation of orchid hybrids in Knudson culture medium with addition of vitamins of MS culture medium, benzilaminopurine and activated charcoal. – Semina: Ciências Agrárias 35(2): 683-694.
- [33] Wang, Y., Li, Z., Huang, L., Su, J. (2013): In vitro mass scale propagation of wild *Cymbidium lowianum* with a rare and endangered plant. – American Journal of Plant Sciences (4): 1500-1507.
- [34] Yadav, A. K., Prasad, Y., Prakash, S., Chand, P., Singh, B. S. G., Singh, G. (2017): Effects of surface sterilization agents on in vitro plant growth in BAPbnana cultivar “Grand Naine”. – IJCS 5(4): 1744-1747.
- [35] Yang, L., Xu, C. J., Hu, G. B., Chen, K. S. (2006): Direct shoot organogenesis and plant regeneration in *Fortunella crassifolia*. – Biol. Plant. 50: 729-732.