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PRODUCTION OF SPOROPHYTIC PLANTS FROM SPORES OF *Nephrolepis exaltata IN VITRO*.

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ABSTRACT

The experiments have been achieved to examine a method to obtain homogenization using gametophytes as well as studying the effect of different culture media composition on sporophyte formation of *Nephrolepis exaltata*. The statistical analysis of data revealed the following findings: Spores were germinated after two weeks at 1/2 strength MS medium supplemented with agar at 1.5 g/L. In the first experiment (multiplication of the gametophytes colonies), the highest significant values of fresh weight (16.19 g) and length (6.02 cm) were obtained after five months as a result of modifying 3/4 strength MS medium with 2 mg/L kinetin + 0.1 mg/L NAA.

In the second experiment in these stages, the highest significant fresh weight of gametophytes colonies value (17.66 g) was obtained as a result of modifying MS medium with 4 mg/L BAP + 0.2 mg/L IAA after three months from spores germination. In sporophytes formation stage, MS 1/2 strength medium supplemented with 3 gm/sucrose + 3 g/L AC induced the highest significant gametophyte colonies diameter (5 cm) in most treatments. However, MS 1/4 strength supplemented with 15 g sucrose recorded the lowest diameter value to about 2.66 cm. As for the percentage of gametophytes colonies showing sporophytes, the combination of MS 1/2 strength medium supplemented with sucrose (15 g/L) significantly increased percentage of gametophytes colonies showing sporophytes formation of about 88%, while gametophytes which were cultured on MS 1/4 strength medium supplemented with sucrose (30 g/L) nearly failed induce sporophytes. In hardening off stage, the highest significant survival percentage of hardened young sporophytes (99.91) was obtained in case of using 2 peat + 2 loam + 1 sand by volume culture medium. The best vegetative growth was reported with 2 peat + 2 loam + 2 sand medium (24.6 cm height, 47.66 fronds and 6.20 g dry weight) per plant. Also the same medium significantly increased values of chlorophyll, total carbohydrates, and total indoles.

It could be concluded through this research, a fast life cycle and high sporophyte production on *Nephrolepis exaltata*, homogenization of gametophytes can be considered to be an excellent method for propagation, yielding incomparable numbers of sporophytes relatively in short period of time.

INTRODUCTION

Nephrolepis exaltata, boston fern, Family Nephrolepidaceae (Oleandraceae), is widely distributed throughout the tropics and subtropics, extending to America, Africa, Asia, Australia, New Zealand and the Island of Pacific. Now, the main economic value of ferns is as popular ornamental foliage plants.

Ferns pass through two distinct phases during their life cycle: the small, simple, haploid gamete-producing phase (gametophyte) and the large, morphologically complex, diploid spore-producing phase (sporophyte). The transition times from gametophyte to sporophyte and vice versa coincide fertilization and sporogensis (Fig. 1) (Fernandez and Revilla, 2003).

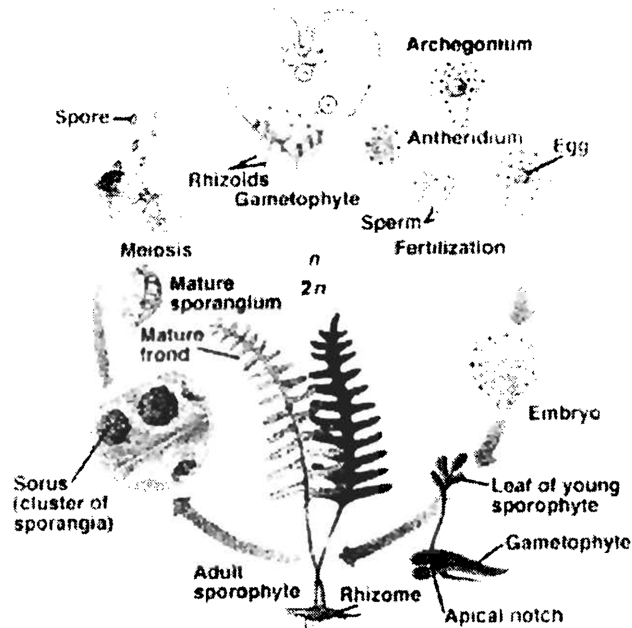

Fig (1) Fern life cycle

Ferns are conventionally propagated by both the sexual as well as the vegetative method. The vegetative method of propagation involves the use of rhizomes or other vegetative organs as planting material. This is a reliable method to produce plants that are genetically identical to the mother plant. New plants of boston fern are generally produced from stolons and tubers isolated from the mother plant. The sexual method of propagation involves raising plants from spores. Linsay (1994) reported a reliable method of propagating fern via spores, considered more advantageous than the vegetative mode of propagation for economic and transport reasons. However, the production of plants from spores depends on several factors such as viability and storage of spores, media and soil surface, sterilization of spores and soil, size of the spore, density of spore sowing, temperature, pH range and gametophyte-sporophyte interaction (Kaur, 1991).

In vitro culture of the fern's life cycle represents a powerful tool for dissecting the mechanisms underlying plant development. This knowledge has important practical repercussion such as to get higher production of species having a great economical value for ornamental industry.

Among various surface sterilizants, the one most; commonly used for ferns has been sodium hypochlorite. The concentration used and length of exposure vary greatly depending on type of plant material. A surfactant such as Tween or an antiseptic soap solution is normally included when some tissues require more exacting sterilization conditions (Fay, 1994). Studies using spores of leptosporangiate ferns have shown that the best germination occurs at slightly acidic or neutral pH (Miller, 1968).

Under natural, conditions, the survival of gametophytes is strongly influenced by the environment. Insufficient moisture or desiccation is an important impediment (Peck *et al.*, 1990). However, under controlled in vitro conditions. gametophyte may be cultured successfully on a variety of media (Fernandez and Reyilla, 2003). Nutrients, as well as other physical and chemical factors such as, light, pH, physical state of medium and plant growth regulators. affect all the processes involved in the growth and development of gametophyte (Hotta and Osawa. 1958; Mohr, 1962; Kato. 1964; Bopp. 1968: Miller, 1968; Swami and Raghavan, 1980; Sheffield and Bell. 1987: Fernandez *et al.*, 1996b, 1997a, 1997b & 1999b). Gametophytes start vegetative reproduction by two means: via gemma or by branching (Fernandez and Revi||a, 2003).

Sporophyte formation occurs by sexual or asexual means. Fernandez *et al.* (1999a) reported some aspects related to sporophyte formation. First of all, differences in the time period from spore culture to sporophyte formation are perceivable among species. Secondly, they have noticed differences among species based on both gametophyte growth and sporophyte formation. He also reported that, the lack of genetic diversity in the gametophytic colonies obtained by asexual reproduction could increase the presence of homozygous individuals and therefore affect their survival in certain species.

The effect of growth substances and nutritional stress on gametophyte regeneration and sporophyte formation was reported by many author's. i.e.. gametophytic cells of *Drypteris affinis* sp. affinis showed a great morphogenetic capacity after their dedifferentiation in the presence of a relatively low auxin/cytokinin ratio, being able to develop different organization patterns, such as callus, gametophyte or sporophyte, as a function of the growth regulators added to the culture medium (Fernandez *et al.*, 1996a). In his report, the culture of the gametophytes during 1 month in the presence of a low auxin/ cytokinin ratio favored sporophyte organization. while gametophyte regeneration was possible after 2 months of culture in a medium supplemented with 6 benzyladenine (BA). Auxin and cytokinin are direct triggers of expression of sporophytic and gametophytic genes in the gametophyte of *D. affinis* sp. affinis: these growth regulators played a key role in at least, the activation of these genes. Kuriyama *et al.* (1990) reported that exogenously supplied BA influenced the gametophyte of *Equisetum arvensis* to produce sporophytes.

A significant increase in sporophyte formation took place after 2 months of culturing gametophytes of *Osmunda regalis* and *Pteris ensitormis* in the absence of nutrients, in a medium containing just water and 0.7 % agar. The sole presence of sucrose in the culture medium, without mineral salts. inhibited gametophyte development of both species, which became necrotic. In *P. ensitorims*, the half-strength MS basal medium without sucrose favored least expansion of sporophytes (Fernandez *et al.*, 1999b).

The first objective of this work was to examine a method to obtain homogenization using gametophytes (multiplication of copies of gametophytes) of *Nephrolepis exaltata.* The second objective was to study the effect of culture medium composition on sporophyte formation. For these

purposes, different concentrations of mineral salts, agar, sucrose, auxins and cytokinins were examined and compared with each other.

MATERIALS AND METHODS

This investigation was carried out during the period between April 2000 - April 2003 at the Tissue Culture Lab., Vegetable and Ornamental Dept., and Greenhouse of the Experimental Station of Faculty of Agriculture, Mansoura Univ.

I.-Spores preparation, sterilization and germination:

Spores of *Nephrolepis exaltata* were obtained from sporophytic plants growing in the greenhouse of the Experimental Station of the Faculty of Agriculture, Mansoura, University. Spores were surface sterilized under aseptic conditions by agitation in ethanol 70% for one minute followed by chlorox at 25% (sodium hypochlorite solution, NaOCL, active gradient 5.25%) with, 0.1% tween-20 (polyoxyethlene sorbitan monolaurate) as a wetting agent for ten minutes. Afterwards, they were rinsed 3 times with sterile distilled water. The spores were sown in test tubes containing 20 ml of 1/2 strength dilution of MS medium (Murashige and Skoog medium, 1962), supplemented with 3% (w/v) sucrose, 1.0 mg/L BAP (6-benzylamino purine) and 0.1 mg/L NAA (ά- Naphthalene Acetic Acid). Medium was subjected to two agar (agar-agar, Sigma Chemical Company) treatment (1.5 or 3 g/L). The media pH was always adjusted to 5.7 prior to agar application. Cultures were incubated at $25 \pm 1^{o}C$ and subjected to 16 hr. light daily by white fluorescent tubes at 40 µE cm^{-2} s^{-1} light intensity).

II. Multiplication of the gametophytes colonies:

Experiment (1).Effect of kinetin and NAA on gametophytes:

Six weeks after germination, colonies of gametophytes were sub cultured on 3/4 strength dilution of MS medium, supplemented with 3% sucrose and 0.6% (w/v) agar. The media pH was always adjusted to 5.7. Medium included four growth regulator treatments (1,2 mg/L. kinetin, 1 kinetin + 0.1 mg/L.NAA or 2 mg kinetin + 0.1 mg/L. NAA.) The medium (30 ml) were dispensed into jars (ca-190 ml). Explants were embedded into the medium and incubated for six weeks after which data on gametophytes fresh weight in (g) were recorded. Fourteen weeks later gametophytes fresh weight and length were recorded.

Experiment (2).Effect of BAP and IAA (Indol Acetic Acid):

Several gametophyte colonies which were growing in 3/4 strength MS medium supplemented with 2 mg/L kinetin and 0.1 NAA showing better results were selected after six weeks and divided into 6 to 8 sections (about 3g. each). Each of these sections were cultured in 1/2 strength MS medium supplemented with (3%) sucrose and (0.6%) w/v agar, and pH was adjusted to 5.7. Medium was divided to nine growth regulator treatments, in mg/L. 1.5 or 3 g/L (1.2,4 BAP, 1 BAP + (0.2 or 0.4 IAA), 2 BAP + 0.2 IAA, 2 BAP +

0.4 IAA, 4 BAP+ 0.2 IAA. or 4 BAP+ 0.4 IAA). Fresh weight (g) and diameter (cm) of gametophyte colonies were recorded after two months.

A complete randomized block design was followed. Each treatment included ten replicates (each of which included 3 jars) in all experiments carried out.

III. Sporophyte plant formation.

A square area of two cm^2 of gametophyte colonies grown in 3/4 strength Ms medium supplemented with 4.0 BAP + 0.2 mg/L. were cultured in the following eight media for two months to stop gametophytes multiplication and to help sporophyte formation. The media were Ms (1/4 or 1/2 strength) combined with sucrose (15 or 30 g/L.) and 3g/L. AC (Activated charcoal) was added or not.

The pH was adjusted to 5.7 prior to agar application (0.6% w/v agar). The media of 30 ml/L. were dispensed to every jar, (ca 90 ml) and autoclaved at 121°C and 1.5 kg cm^{-2} for 20 minutes. Cultures were incubated for three months at 25±1°C and subjected to 16 hours light daily by white fluorescent tubes at 40 μE cm^{-2} s^{-1} light intensity.

Data recorded

Diameter (cm) of gametophytes colonies, percentage of gametophytes showing sporophyte plants, length (cm) of sporophyte plants, number of fronds / sporophyte plant.

The eight experimental treatments were arranged in a split-split design with ten replicates, each of which included 3 jars. The nutrient media were considered as main plots (i.e. 1/4 or 1/2Ms. strength), 15 or 30 g/L sucrose concentrations were the sub- plots, and with or without AC. (Activated charcoal), were the sub- sub- plots.

IV. Hardening-off stage (Acclimatization):

The developed young sporophytes were transferred from the jars and washed with tap water to obtained agar free sporophytes. The plantlets were transferred for three months into 0.2 liter capacity plastic pots containing mixtures of 1peat moss +1 loam +1 sand, 2 loam + 1 sand, 2 peat +1 sand, 2 peat + 2 loam +1 sand in which they lasted for six weeks .

Acclimatization of the developed plantlets was carried out under mist in a greenhouse, changing humidity gradually from 90 to 60% over one month period.

Fifteen plantlets, in three replicates were used, and the pots were distributed in complete randomized design. At the end of the Acclimatization stage the following parameters were recorded:-

Survival capacity (%) – length of fronds and weight of plants.

The acclimatized plants were subjected to chemical analysis as follows: fifteen grams of fresh samples in three replicates were extracted with 80% cold methanol, then the following endogenous substances were quantitized by using spectrophotometric measurements of tissue extracts and estimated as mg/ 100 g f. w.:

- Total soluble carbohydrates according to Dubois *et al.* (1966).

- Total indoles according to Larsen *et al.* (1962) and modified by Selim *et al.* (1978).
- Total soluble phenols according to AOAC (1985).
- Chlorophyll was measured by A Minoltc SPAD chlorophyll meter in units (Yadova, 1986).

Data were subjected to analyses of variance according to Steel and Torrie, (1980). Mean separation was made using least significant difference (L.S.D. at 5% level of significance). In case of percentages, the original data were arcsine – transformed prior statistical analysis.

RESULTS AND DISSECTION

I. Spores germination:

Effect of different agar concentrations:

Spores of *Nephorolepis exaltata* first germinated (evaluated as greening) after two weeks in 1/2 strength MS medium supplemented with 1.5 g/L agar (Fig 2). It should be noted herein that the other medium including more agar content i.e. (3 g/L) failed absolutely to show any greening. So, it was obvious that lowering the agar content was advisable in this respect.

Microscopic examination of the gametophyte colonies were green in colour. A spherical velvet shape threads were detected.

Fig (2). Gametophytes clumping were observed.

II. Multiplication of the gametophytes colonies:

1- Effect of kinetin and NAA on gametophytes growth:-

Data represented in Figs (3 and 3) illustrate the significant enhancing effect of kinetin and NAA on gametophytes fresh weight (g) and length (cm) after 20 weeks from sub culturing the colonies of gametophytes. The highest significant values of fresh weight (16.19 g) and length (6.02 cm) were obtained as a result of modifying 3/4 MS strength medium with 2 mg/L kinetin + 0.1 mg/L NAA.

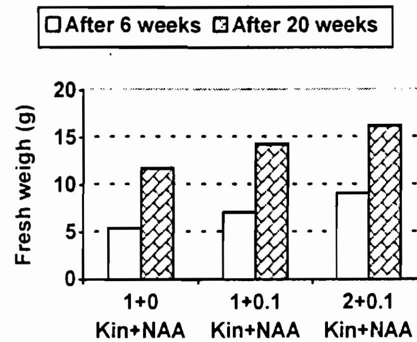

Fig. 3. Effect of kinetin and NAA on gametophytes fresh weight (g) after 6 and 20 weeks. (LSD at 5% = 1.31 (after 6 weeks) and 0.71 (after 20 weeks).

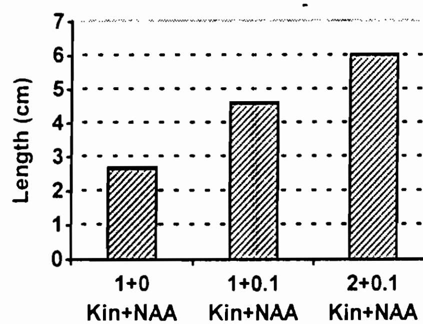

Fig. 4. Effect of kinetin and NAA on gametophytes length (cm) after 20 weeks. (LSD at 5% = 0.71).

These results might be ascribed to the effective role played by the cytokinins (kinetin) and auxins (NAA) in the micro propagation of plants. Cytokinins and auxins have been shown to stimulate cell division, as well as, cell elongation and to activate RNA synthesis and to stimulate protein synthesis and enzyme activity resulting in increasing fresh weight and length.

2- Effect of BAP and IAA on gametophytes fresh weight after two months:-

Data represented in Table (1) illustrate the enhancing effect of plant growth regulators on gametophytes fresh weight. The highest fresh weight of gametophytes (15.76 g) resulted with BAP at 4 mg/L compared with other two BAP levels. On the other hand, IAA at its two levels had significant effect in this concern comparing with the control.

As for the interaction effect of the two growth regulators on fresh weight of the gametophytes, it was clear from data at the same table that, fresh weight of boston fern gametophytes increased with all treatments at

different levels. The highest significant fresh weight value (17.66 g) was obtained as a result of modifying MS medium with 4 mg/L BAP + 0.2 mg/L IAA, that made about 2.39 times of fresh weight when compared to control treatment (1mg/L BAP +0 IAA).

Table (1): Effect of different concentrations of BAP and IAA on fresh weight (g) of gametophytes colonies after 2 months.

IAA mg/L / BAP mg/L	0	0.2	0.4	Means of BAP
1	7.36	8.97	9.92	8.75
2	9.99	10.76	12.98	11.24
4	14.15	17.66	15.48	15.76
	10.50	12.46	12.75	
LSD. at 5 %	IAA = 0.08 BAP= 0.08 IAA x BAP = 1.35			

These results might be explained by the assumption -that the externally applied cytokinin BAP at 4 mg/L and auxin IAA at 0.4 mg/L achieved an internal hormonal balance that induced the highest cell differentiation into vegetative organ. This enhancing effect could be ascribed to the stimulating effect of these concentrations of BAP as a cytokinen and IAA as an auxin, on cell division on the expense of cell enlargement. Moreover, cytokinin have been shown to activate RNA synthesis and to stimulate protein synthesis and enzyme activity.

Gametophytes undergo vegetative reproduction by branching, since a new gametophyte begins as a one, dimensional filament that soon becomes two – dimensional. Subsequently taking on the typical shape of gametophytes produced from spores Fig. (5). This is the most frequent way for gametophytes to propagate themselves *in vitro*, (Fernandez and Revilla, 2003).

Fig. (5): Vegetative reproduction of gametophyte colonies.

III. Sporophytes formation

1- Diameter (cm) of gametophytes colonies.

Regarding to the effect of MS medium strength, sucrose concentration and activated charcoal (AC) on gametophyte colonies diameter, data recorded in Table (2) showed that 1/2 strength dilution of MS medium increased gametophyte colonies diameter by 16% than 1/4 strength MS. Also, applying 30 g sucrose to the culture medium increased gametophyte colonies diameter by 19% than 15 g sucrose, but the differences were not significant.

Concerning the effect of AC, data at the same table disclosed that applying AC to the culture medium significantly increased gametophyte colonies diameter by about 24% than cultures without AC applying.

Table 2: Effect of MS strength medium (A), sucrose concentration (B) and activated charcoal (C) on gametophytes colonies diameter (cm).

A	B \ C	0 mg/L	3 mg/L	A	B
1/4	15	2.66	4.00		
	30	4.16	4.60	3.85	3.79
1/2	15	3.66	4.83		
	30	4.30	5.00	4.45	4.52
	C	3.70	4.60		
LSD at 5%		A=NS B=NS C=0.77 A x B x C= 1.51			

Moreover, it was indicated that the presence of activated charcoal (AC) significantly increased gametophyte colonies diameter compared with control (without AC). The beneficial effects of activated charcoal may be from the darkening of the medium which stimulated the natural soil characteristics. In addition, Klein and Bopp (1971), reported that activated charcoal has been used as a light adsorbance in agar nutrient substrates to prevent light which induced growth inhibition of tissues. Moreover, they reported that some plants have the unpleasant characteristics of exudating brown / black pigments upon wounding (usually oxidized poly phenol, like compound and tannins), which often making growth and development impossible. Fridborg and Eriksson (1975) also reported that activated charcoal adsorb gases and perhaps stimulate growth of injured explants probably due to ethylene absorption.

As for the interaction effect of MS medium strength, sucrose concentration and AC content on gametophyte colonies diameter, data at the same table indicated that MS- half strength supplemented with 30 g sucrose and 3 g AC induced the highest gametophyte colonies diameter value about (5 cm). However, MS quarter strength supplemented with 15 g sucrose without AC addition recorded the lowest diameter value (2.66 cm). The significant increment difference between the two media was about 88%. These results indicate that the first medium involved higher concentration of

inorganic salts, sucrose and AC, hence, these factors are obviously crucial for the needs of the explants used in order to multiplicate. Supporting results were reported by Fernandez *et al.*, (1999a) with *Osmuncla regalis* and *Pteris ensiformis* plants.

2- Percentage of gametophytes colonies showing sporophytes as affected by MS strength medium (A), sucrose concentration (B), and activated charcoal (C).

It was obvious that medium strength highly influenced percentage of sporophytes formation. From Table (3), it is clear that MS half strength medium surpassed its quarter strength effect with the highest value (36.6%) on sporophytes formation (Fig 6).

Concerning the effect of sucrose concentration on percentage of sporophytes formation, as revealed from Table (3) applying low sucrose concentration (15 g), augmented sporophytes formation with the highest significant value (55%) compared to modifying the medium by the higher sucrose concentration (30 g). Moreover, the culture medium without AC application significantly increased sporophyte formation (34.9%) than which contained 3 g AC (16%).

The combination of MS-half strength medium supplemented with sucrose (15 g/L) played an important task on sporophyte formation percentage which produced (88%) that makes about 883 times of sporophyte formation percentage when compared with those which formed at MS- quarter strength supplemented with sucrose at 30 g/L. Also, it became about 5.4 times of sporophyte formation percentage when compared with those which formed at MS-half strength supplemented with sucrose at 30 g/L.

Table 3: Percentage of gametophyte colonies showing sporophyte as affected by MS strength medium (A), sucrose concentration (B), and activated charcoal (C), after 5.5 months from spores germination.

A	B \ C	mg/L 0 Arc $\sqrt{\%}$	0 %	3 Arc $\sqrt{\%}$	3 %	A Arc $\sqrt{\%}$	A %	B Arc $\sqrt{\%}$	B %
1/4	15	54.60	66.5	35.17	33.2				
	30	00.57	00.1	00.75	00.1	22.70	14.9	47.58	55
1/2	15	65.82	88.3	34.75	32.5	37.21	36.6	12.26	4.5
	30	23.86	16.3	24.04	16.6				
Mean C		36.21	34.9	23.63	16.0				
L.S.D. at 5%		A=0.69	B=1.43	C=1.43		A x B x C= 2.85			

Are $\sqrt{\%}$ = Arcsine $\sqrt{percentage}$ transformation.

Fig. (6): Production of sporophytes (SP) still attached to the gametophyte (GP) after 22 weeks from spores germination.

Dealing with data in Tables (2 ad 3) it could be concluded that, when *Nephrolepis exaltata* gametophytes were cultured in MS-half strength medium containing low sucrose concentration (15 g/L) without AC addition for two months, a significant increase in sporophytes formation took place. Low sucrose concentration and absence of AC in the culture medium, inhibited gametophyte development.

It worthies to indicate that the excellent multiplication capacity observed in *Nephrolepis exaltata* gametophytes were when cultured *in vitro* was in contrast to the low sporophyte formation rate Analogous suggestion was reported by Fernandez *et al.* (1999a) in *Petris ensiformis* whom explained that the lack of genetic diversity in the gametophytic colonies by undergoing a sexual reproduction could increase the presence of homozygous individuals and therefore affect their survival. The second explanation supposes a nutritional competence between gametophytes and sporophytes. Embryo formation occurs near to the apical notch and gametophyte multiplication takes place in the basal region, the nutrients present in the culture medium are taken up by the rhizoids, in the basal region, to supply a great number of gametophytes, so sporophyte formation could be affected.

IV. Hardening-off stage (Acclimatization):

In acclimatization stage a significant effects of different various media mixtures on survival capacity %, growth characters and chemical composition of the *ex vitro* plantlets were showed in Tables (4 and 5).

Effect of culture media mixtures on behavior of harding of stage of young sporophytes *ex vitro* (acclimatization).

In hardening of stage a significant effects of various culture media mixtures on survival capacity (%), growth characters and chemical composition of the *ex vitro* young sporophytes were shown in Tables (4 and 5).

A- Survival capacity and growth of young sporophytes *ex vitro*.

It was evident from data in Table (4) and Fig (7) that the highest survival percentage of the hardeneal young sporophytes (99.91) was obtained in case of using 2 peat + 2 loam + 1 sand (by volume) culture media. While, the lowest value (50.16) was recorded for 2 loam + 1 sand culture medium. This was followed by the survival percentage of (96.6) which was found when 1 peat + 1 loam + 1 sand culture medium was used. However the difference between the two mixtures was significant.

Table (4): Effect of growing media mixtures on the behavior of acclimatization stage of *Nephrolepis exaltata* plantlets *ex vitro*.

Measurements / Treatments			Survival Arc $\sqrt{\%}$	Survival %	Plant length (cm)	No. of fronds/plant	Plant dry weight (g)
Peat	loam	sand					
1	1	1	79.46	96.60	24.00	42.33	5.96
0	2	1	45.08	50.16	14.16	18.33	4.26
2	0	1	66.86	84.90	18.16	29.66	5.32
2	2	1	88.29	99.91	24.66	47.66	6.20
LSD at 5%			3.53		2.77	3.46	0.17
LSD at 1%			5.35		4.20	5.25	0.30

Arc $\sqrt{\%}$ = Arcsine $\sqrt{percentage}$ transformation.

Table (5): Effect of growing media mixtures on chemical composition of *Nephrolepis exaltata* plantlets *ex vitro*.

Components / Treatments			Chlorophyll SPA units	Total carbohydrates mg/100g F.W.	Total indoles mg/100g F.W.	Total phenols mg/100g F.W.
Peat	loam	sand				
1	1	1	68.34	116.00	895.33	221.00
0	2	1	51.72	131.66	420.00	137.00
2	0	1	58.53	140.00	530.66	184.00
2	2	1	72.42	170.00	1025.00	248.00
LSD at 5%			10.66	13.56	47.92	14.49
LSD at 1%			16.17	20.58	72.71	22.05

This decrease in survivals may be related to less moisture available essential for growth. Concerning the effect of different growing culture media on sporophyte length, No. of fronds sporophyte and sporophyte dry weight (g). data at the same table showed that the lowest values (14.16 cm height, 18.33 fronds and 4.26 dry weight) were recorded from 2 loam + 1 sand medium. On the other hand, results showed that the other growing media significantly improved vegetative growth compared with 2 loam + sand medium. The best vegetative growth in this matter was reported with 2 peat + 2 loam + 1 sand medium (24.66 cm height, 47.66 fronds and 6.20 g dry weight per plant).

From the previous results it could be concluded that the mixture of peat + loam + sand had an effect on increasing survival % and vegetative growth of sporophytes at any volume used in this concern.

The mixing of sand with loam and organic peat (organic matter) increases water holding capacity beside adding a wide range of fertilizer elements through loam and peat. Though, usually nutrients are present in small amounts but resulted in vegetative growth and survival % improvement. It should be also noted that aeration level which is important for root growth and respiration may became more ideal in such mixture.

Fig. (7): Healthy growth in mixture of 2 peat+ 2 loam+ 1 sand after 7 months from spores germination.

B- Chemical composition of young sporophytes *ex vitro*.

The different mixtures of growing media tested caused mostly high significant influences on the endogenous level of chlorophyll, total carbohydrates, total indoles and total phenols in the ex vitro young sporophytes tissues (Table 5).

Since, data recorded in Table (5) indicated that chlorophyll content reached minimum level (51.72 SPA units) at growing medium of (2 loam + 1 sand), while the addition of peat and loam to the culture media increased significantly chlorophyll content when compared with media without peat or loam. These results may be related to the increase in availability of nitrogen, magnesium and other elements resulted from adding peat moss and loamy soil leading to the increase in the biosynthesis of chlorophyll pigment. These findings were confirmed with those of Mansour *et al.* (1994) on *Syngonium podophyllum*.

Dealing with the effect of different growing media in total carbohydrates, in the same table indicated that peat, loam, and sand media significantly increased total carbohydrates compared with media without peat or loam.

This may be logically true, since the rate of photosynthesis processes was increased as a result of the increment in leaves content of chlorophyll and consequently total carbohydrates were increased. Regarding, total

indoles and phenols, the highest amounts (1025 and 248 mg/100 g FW.) respectively, were determined in sporophytes cultured in (2 peat+ 2 loam+ 1 sand v/v) medium. While, the lowest values ranged from 420 to 895.33 for indoles and 137 to 221 mg/100 g FW for phenols resulted from the other tested growing media.

The increments in the different parameters analysed means clearly that metabolically activates took place.

It may be concluded that, *in vitro* culture of the fern's life cycle represents a powerful tool for dissecting the mechanisms underlying plant development. To deep on phase change in this plant group, or gametophyte-sporophyte transition, would throw light to know about the complex rules of plant physiology by means of easer and accurated experimental systems. This knowledge has important practical repercussions such as to get higher production of species having a great economical value for ornamental industry.

The derived results from this research showed clearly, the possibility of inducing an incredible number of plants through the application of these methods in other ferns having a great economical value of ornamental industry. Tree ferns (*Cyathea spp.*), Ostrich fern (*Mattcuccia struthia* pteris) and Stag's horn fern (*Platycerium alcicorne*) are examples of such plants which give in common just a limited divisions for propagation purposes.

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إكثار نبات الفوجير بواسطة الجراثيم باستخدام مزارع الأنسجة

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أجرى هذا البحث بمعمل زراعة الأنسجة بقسم الخضر والزينة والصوبة الزجاجية بمحطة الأبحاث بكلية الزراعة – جامعة المنصورة خلال الفترة من ابريل ٢٠٠٠ حتى ابريل ٢٠٠٣، بهدف إكثار نبات الفوجير بالجراثيم باستخدام تكنيك زراعة الأنسجة، وذلك عن طريق إنبات الجراثيم ثم تقسيم وإكثار الجاميطات المتجانسة (homogeneous gametophytes) ثم دفع هذه الجاميطات عن طريق بعض المعاملات لإنتاج أعداد كبيرة من نبات الفوجير (sporophytes) في فترة قصيرة.

ومن أهم النتائج المتحصل عليها بعد التحليل الإحصائي ما يلي:-

١- في مرحلة إنبات الجراثيم spores germination – تمت عملية الإنبات بظهور نقط خضراء اللون (الجاميطات) بعد أسبوعين من زراعة الجراثيم على بيئة موراشيج وسكوج (نصف قوة) مضاف إليها ١،٥ جم/لتر أجار.

٢- مرحلة تضاعف وإكثار مستعمرات الجاميطات (Multiplication of the gametophytes colonies)، تم الحصول على أعلى قيم معنوية لكل ن الوزن الطازج للمستعمرات الجاميطية (١٦،١٩جم) وأطول جاميطات (٦،٠٢سم)، عندما زرعت أجزاء من المستعمرات الجاميطية على بيئة موراشيج وسكوج (٣/٤ قوة) مضاف إليها ٢مجم كينتين+ ٠،١مجم نفتالين حمض الخليك/ لتر، بعد ٥ شهور من إنبات الجراثيم.

٣- وفى تجربة أخرى أخذت أجزاء من جاميطات التجربة السابقة بعد ٦ أسابيع وتم زراعتها على بيئات تحتوى على تركيزات مختلفة من منظمات النمو، وكانت أعلى قيمة معنوية للوزن الطازج للمستعمرات الجاميطية (١٧،٦٦جم) وذلك بإضافة ٤جم بنزايل أمينو بيورين+ ٠،٢مجم أندول حمض الخليك لكل لتر من بيئة موراشيج وسكوج وذلك بعد شهرين من الزراعة.

٤- مرحلة إنتاج نباتات الفوجير (sporophytes formation)
- تم الحصول اقل قطر للمستعمرات الجاميطية (٢،٦٦ سم) باستخدام بيئة موراشيج وسكوج (١/٤ قوة) مضاف إليها ١٥جم سكروز فقط /لتر من البيئة، بينما تم الحصول على أعلى قطر (٥ سم) باستخدام بيئة (١/٢ قوة) مضاف إليها ٣٠جم سكروز+ ٣جم فحم نشط / لتر بيئة.
- أنتجت المستعمرات الجاميطية المنزرعة على بيئة (١/٤ قوة) مضاف إليها ١٥جم سكروز / لتر بيئة أعلى نسبة مئوية من نباتات الفوجير حيث بلغت ٨٨ % وذلك بعد شهرين من الزراعة.
- انخفضت النسبة إلى ٠،١ % فقط عند استخدام بيئة (١/٢ قوة) مضاف إليها ٣٠جم سكروز لكل لتر من البيئة.

٥- مرحلة الأقلمة Hardening-off (Acclimatization)
- ارتفعت معنوياً قدرة نباتات الفوجير على التأقلم للمعيشة في الوسط الخارجي حيث وصلت إلى ٩٩،٩% ووصل طول النبات إلى أقصى إرتفاع (٢٤،٦٦سم)، وكذلك أكبر عدد من الأفرع (٤٧،٦٦ فرع)، وأكبر قيمة للوزن الجاف للنبات (٦،٢ جم)، بعد ٦اسابيع من الزراعة على بيئة مكونة من بيت موس+ طمي+ رمل بنسبة ٢:٢: ١ حجماً، حيث كانت الزيادة معنوية عن باقي البيئات المختبرة.
- كذلك أنتجت نفس البيئة السابقة أعلى قيم معنوية لكل من الكلوروفيل (لمحتوى أوراق النباتات المؤقلمة) والكربوهيدرات والفينولات والاندولات لمحتوى أنسجة النباتات.

وبذلك أمكن الحصول على نباتات الفوجير مؤقلمة وبأعداد وفيرة للغاية في فترة ٧ شهور ونصف من بداية زراعة الجراثيم، كما يمكن تطبيق هذه الطريقة لإنتاج وإكثار السرخسيات النادرة والمرتفعة القيمة الاقتصادية تحت ظروف التجربة.