THE MORPHOGENESIS OF *Nephrolepis exaltata* Schott VITROCULTURES PREVAILED FROM STOLON APEXES, CULTIVATED ON ASEPTIC MEDIA WITH AUXINE CONTENT

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Abstract

This study establishes the influence of various concentrations of auxine on Nephrolepis exaltata Schott vitrocultures. The β indolilbutiric acid (AIB) and α naftilacetic acid (ANA), as growing substances, determine an intensification risogenesis, phylogenesis and calusogenesis at the level of explants prevailed from stolon apexes. Concentrations of 1 mg/l, 1.5 mg/l and 2 mg/l β indolilbutiric acid (AIB) and α naftilacetic acid (ANA), in Murashige-Skoog culture media (1962) [11] influence morphogenesis and organogenesis in a positive way at the level of Nephrolepis exaltata Schott explants.

Keywords: auxine, growing substance, *Nephrolepis exaltata* Schott

INTRODUCTION

The artificial vegetative multiplication at *Nephrolepis* can be done by cutting, using primary or secondary stolons (Zamora, 2000). The stolons are capable of organogenesis, and can produce new plants after the *in vitro* multiplication. The best culture media used for the *in vitro* multiplication of ferns, recommended by other researchers was ½ Murashige-Skoog (1962) (Ambrósio and de Melo, 2004; Fernández and Revilla, 2003; Hegdel et al., 2006; Martin et al., 2006; Somer et al., 2009).

The present study is desired to be a confirmation of the researches, meant to follow the reaction of *Nephrolepis exaltata* Schott stolon apexes in those cultivation conditions in aseptic, solid culture medium, in the presence of growth regulators (Pop Maghiar, 2012). The evolution of the stolon apexes was followed for 90 days, their reaction being studied in dependence to the presence of the hormone AIB and ANA composition of the culture medium. At the stolon level it has been overviewed the estimation of the multiplication and organogenesis process in a period of 3 months.

MATERIAL AND METHODS

In the above mentioned experiment, the methodological particularities used for the *in vitro* multiplication of *N. exaltata* Schott stolon apexes consisted of various concentrations of β indolilbutiric acid (AIB) and α

naftilacetic acid (ANA), respectively 1, 1.5 and 2 mg/l, used in Murashige-Skoog (Murashige and Skoog, 1962) culture media, in 6 experimental variants. The bio-measures were carried out at 30, 60 and 90 days of inoculation, on 6 parameters, the average values and their state.

After inoculation, the containers with inoculi were passed to the growing room, exposed in a white fluorescent light with an intensity of 1700 lux and a photoperiod of 16 hours of light per day (Pessoa et al., 2004). The ambient temperature varied between 24-26˚C during the day and about 22˚C at night. Other researchers noticed that vitroplantlets are formed and develop better in the conditions of their growing in the thermic regime of 25˚C ± 2˚C and a photoperiod of 16-18 hours of light per day (Lazar et al., 2010; Torres, 1989). A single stolon apex was introduced in each culture container, and for each experimental variant 50 culture containers were inoculated. The culture media used for inoculation contained β indolilbutiric acid (AIB) and α naftilacetic acid (ANA) auxines, as adding to the basic medium, in 6 variants:

V_0- Murashige-Skoog (1962) (MS) basic medium (MB) without growing regulators –witness lot;

V_1- MS-MB with adding of 1 mg/l β indolilbutiric acid (AIB);

V_2- MS-MB with 1.5 mg/l AIB;

V_3- MS-MB with 2 mg/l AIB;

V_4- MS-MB with 1 mg/l α naftilacetic acid (ANA);

V_5- MS-MB with 1.5 mg/l ANA;

V_6- MS-MB with 2 mg/l ANA.

The experimental data obtained at the control variant, respectively on V_0 variant basic medium (MB-MS complete, without growth regulators) was considered as reference lot (control), respectively 100%, the average of the registered values – to each parameter and variant – fractionally – were reported to the average values obtained for the similar parameters, to the witness variant. The experimental dates were statistically processed; establishing – based on the variability values – the sense of these. The most representative aspects were presented and discussed in the analysis part of the experimental results (Fig. 1-3).

RESULTS AND DISCUSSION

In the biometric data registered at vitrocultures of *N. exaltata* Schott (Fig. 1-3), at the culture media that contain β indolilbutiric acid (AIB), in different concentrations, the following aspects can be mentioned:

- at 60 days from the initiation of cultures, risogenesis was present at all experimental variants used, process that increased as time went by, during the 90 days of vitroculture; the most prolific variant – as regards risogenesis - was that which contained in the culture media the biggest

amount of AIB, respectively 2 mg/l (V_3), at the level of explants it had 4 roots regenerated with an average lengths of 0,45 cm (Table 2-3);

- at 30 and 60 days from the explants inoculation at the level of inoculi a maximum of 4 leaves was regenerated with an average length of 1 cm (Tables 1-2), at all variants of culture media that contained auxin AIB in various concentrations between 1 mg/l and 2 mg/l; but, at 90 days of vitroculture those 5 leaves formed at the apical area of inoculi a maximum average length of 2 cm at variants that contained 1, respectively 1,5 mg/l AIB (table 3), as compared to the variant that contained in the culture media 2 mg/l AIB (V_3), for which the explants regenerated 5 leaves with a maximum average length of 3 cm (Table 3);

- at 30 and 60 days of vitroculture (Table 1-2), at all experimental variants that contained AIB the calusogenesis was present, with an exception registered in observations done in the 60th day, at the variant that contains 1 mg/l AIB (V_1**)**; at 90 days from the incubation of explants the calusogenesis was not present (Table 3).

In the biometric data registered at the explants of *N. exaltata* Schott (Fig. 1-3), at the culture media that contain *α naftilacetic acid* (ANA), in different concentrations, the following conclusions can be mentioned:

- at all variants of culture media which contained auxin ANA, the risogenesis was present even from the first 30 days from the initiation of experiments, process that increased as time went by, during the 90 days of vitroculture, when, at the level of inoculi 4 roots were regenerated with an average length of 0,75 cm (Table 1-3);

- filogenesis is present from the first 30 days of vitroculture, at all variants that contain auxin ANA, after this process there were cultures at which the maximum average length of the only leaf regenerated was of 1 cm (table 1); only at biomeasurements made at 90 days from the initiation of vitrocultures, the explants cultivated on the environment variants that contained 1 mg/l ANA (V_4), 1,5 mg/l ANA (V_5) and respectively 2 mg/l ANA (V_6) formed a maximum of 5 leaves with the maximum average length of de 2 cm (Table 3);

- as it was noticed at the vitrocultures of *N. exaltata* Schott, inoculated on culture media that contain auxines AIB and ANA with concentrations between 1 and 2 mg/l, at this first biomeasurement the length of the regenerated leaves at the level of miniplants was of 1 cm too (Table 1-2);

- as the explants cultivated on media with AIB, at the explants of *N. exaltata* Schott, cultivated on media with auxin ANA the phenomenon of calusogenesis was present (Table 1), significant from the first 30 days of inoculation of stoloniferous apex inoculi, calus that regenerated on its area leaves up to 60 days from the initiation of the experiment (Table 2).

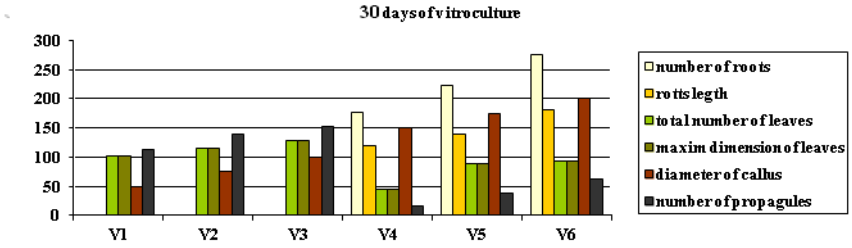

Figure 1. Biometric data regarding the growth of *Nephrolepis exaltata* Schott vitro-plantlets after *30 days* of vitroculture, in the following variants: $\mathbf{V_1}$- MB with 1 mg/l *β indolilbutiric acid* (AIB), $\mathbf{V_2}$- MB with 1.5 mg/l AIB, $\mathbf{V_3}$- MB with 2 mg/l AIB, $\mathbf{V_4}$- MB with 1 mg/l *α naftilacetic acid* (ANA), $\mathbf{V_5}$- MB with 1.5 mg/l ANA, $\mathbf{V_6}$- MB with 2 mg/l ANA, compared to the parameters recorded at the level of vitro-plantlets from the witness lot ($\mathbf{V_0}$), values considered 100%.

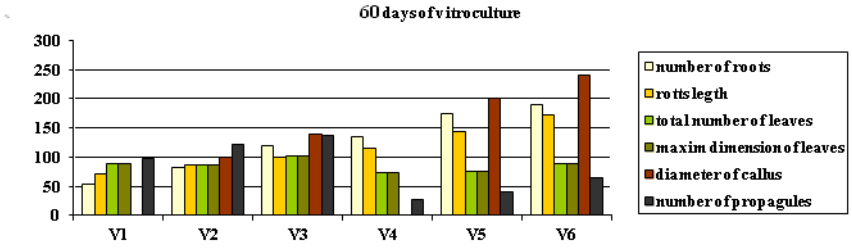

Figure 2. Biometric data regarding the growth of *Nephrolepis exaltata* Schott vitro-plantlets after 6*0 days* of vitroculture, in the following variants: $\mathbf{V_1}$- MB with 1 mg/l *β indolilbutiric acid* (AIB), $\mathbf{V_2}$- MB with 1.5 mg/l AIB, $\mathbf{V_3}$- MB with 2 mg/l AIB, $\mathbf{V_4}$- MB with 1 mg/l *α naftilacetic acid* (ANA), $\mathbf{V_5}$- MB with 1.5 mg/l ANA, $\mathbf{V_6}$- MB with 2 mg/l ANA, compared to the parameters recorded at the level of vitro-plantlets from the witness lot ($\mathbf{V_0}$), values considered 100%.

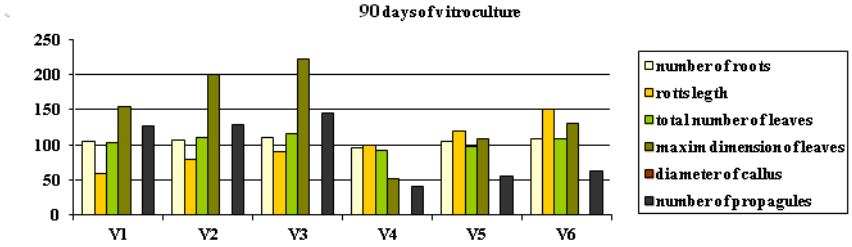

Figure 3. Biometric data regarding the growth of *Nephrolepis exaltata* Schott vitro-plantlets after 9*0 days* of vitroculture, in the following variants: $\mathbf{V_1}$- MB with 1 mg/l *β indolilbutiric acid* (AIB), $\mathbf{V_2}$- MB with 1.5 mg/l AIB, $\mathbf{V_3}$- MB with 2 mg/l AIB, $\mathbf{V_4}$- MB with 1 mg/l *α naftilacetic acid* (ANA), $\mathbf{V_5}$- MB with 1.5 mg/l ANA, $\mathbf{V_6}$- MB with 2 mg/l ANA, compared to the parameters recorded at the level of vitro-plantlets from the witness lot ($\mathbf{V_0}$), values considered 100%.

Table 1

Comparative aspects regarding the reactivity of vitroplantlets of *Nephrolepis exaltata* Schott cultivated on modified Murashige–Skoog (1962) media (MB), with a varied content of growing regulators, where: **V_0**- MB with no growing regulators; **V_1**- MB with adding of 1 mg/l *β indolilbutiric acid* (AIB); **V_2** - MB with adding of 1.5 mg/l AIB; **V_3** - MB with adding of 2 mg/l AIB; **V_4** - MB with adding of 1 mg/l *α naftilacetic acid* (ANA); **V_5** - MB with adding of 1.5 mg/l ANA; **V_6** - MB with adding of 2 mg/l ANA, at *30 days* from inoculation.

Biometrics / Statistics evaluation	Number of roots	Roots length	Total number of leaves	Maxim dimension of leaves	Diameter of callus	Number of propagules
Type V_0						
$\bar{x} \pm S_{\bar{x}}$	0.17 ± 0.15	0.25 ± 0.12	1.17 ± 0.11	1.17 ± 0.11	0.20 ± 0.19	0.66 ± 0.10
s	0.0225	0.0144	0.0121	0.0121	0.0361	0.0100
S%	100%	100%	100%	100%	100%	100%
Type V_1						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.19 ± 0.13	1.19 ± 0.13	0.10 ± 0.48	0.75 ± 0.11
s	-	-	0.0169	0.0169	0.2304	0.0121
S%	-	-	101.71%	101.71%	50%	113.63%
Type V_2						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.34 ± 0.22	1.34 ± 0.22	0.15 ± 0.32	0.92 ± 0.21
s	-	-	0.0484	0.0484	0.1024	0.0441
S%	-	-	114.53%	114.53%	75%	139.39%
Type V_3						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.49 ± 0.28	1.49 ± 0.28	0.20 ± 0.19	1 ± 0.32
s	-	-	0.0784	0.0784	0.0361	0.1024
S%	-	-	127.35%	127.35%	100%	151.51%
Type V_4						
$\bar{x} \pm S_{\bar{x}}$	0.30 ± 0.26	0.30 ± 0.20	0.51 ± 0.31	0.51 ± 0.31	0.30 ± 0.25	0.10 ± 0.89
s	0.0676	0.0400	0.0961	0.0961	0.0625	0.7921
S%	176.47%	120%	43.59%	43.59%	150%	15.15%
Type V_5						
$\bar{x} \pm S_{\bar{x}}$	0.38 ± 0.30	0.35 ± 0.40	1.04 ± 1.07	1.04 ± 1.07	0.35 ± 0.41	0.25 ± 0.68
s	0.0900	0.1600	1.1449	1.1449	0.1681	0.4624
S%	223.53%	140%	88.89%	88.89%	175%	37.87%
Type V_6						
$\bar{x} \pm S_{\bar{x}}$	0.47 ± 0.65	0.45 ± 0.80	1.10 ± 1.12	1.10 ± 1.12	0.40 ± 0.88	0.41 ± 0.20
s	0.4225	0.6400	1.2544	1.2544	0.7744	0.0400
S%	276.47%	180%	94.01%	94.01%	200%	62.12%

Note: $\bar{x} \pm S_{\bar{x}}$ (average ± standard deviation of the average), s (standard deviation), S% (variability coefficient).

Table 2

Comparative aspects regarding the reactivity of vitroplantlets of *Nephrolepis exaltata* Schott cultivated on modified Murashige–Skoog (1962) media (MB), with a varied content of growing regulators, where: **V_0**- MB with no growing regulators; **V_1**- MB with adding of 1 mg/l *β indolilbutiric acid* (AIB); **V_2** - MB with adding of 1.5 mg/l AIB; **V_3** - MB with adding of 2 mg/l AIB; **V_4** - MB with adding of 1 mg/l *α naftilacetic acid* (ANA); **V_5** - MB with adding of 1.5 mg/l ANA; **V_6** - MB with adding of 2 mg/l ANA, at *60 days* from inoculation.

Biometrics / Statistics evaluation	Number of roots	Roots length	Total number of leaves	Maxim dimension of leaves	Diameter of callus	Number of propagules
Type V_0						
$\bar{x} \pm S_{\bar{x}}$	1.26 ± 0.15	0.35 ± 0.20	4.28 ± 0.16	4.28 ± 0.16	0.05 ± 0.20	0.79 ± 0.35
s	0.0225	0.0400	0.0256	0.0256	0.0400	0.1225
S%	100%	100%	100%	100%	100%	100%
Type V_1						
$\bar{x} \pm S_{\bar{x}}$	0.66 ± 0.48	0.25 ± 0.28	3.83 ± 0.06	3.83 ± 0.06	-	0.77 ± 0.30
s	0.2304	0.0784	0.0036	0.0036	-	0.0900
S%	52.38%	71.43%	89.48%	89.48%	-	97.46%
Type V_2						
$\bar{x} \pm S_{\bar{x}}$	1.03 ± 0.18	0.30 ± 0.14	3.70 ± 0.05	3.70 ± 0.05	0.05 ± 0.20	0.96 ± 0.37
s	0.0324	0.0196	0.0025	0.0025	0.0400	0.1369
S%	81.74%	85.71%	86.45%	86.45%	100%	121.52%
Type V_3						
$\bar{x} \pm S_{\bar{x}}$	1.50 ± 0.19	0.35 ± 0.00	4.38 ± 0.17	4.38 ± 0.17	0.07 ± 0.27	1.08 ± 0.41
s	0.0361	0	0.0289	0.0289	0.0729	0.1681
S%	119%	100%	102.33%	102.33%	140%	136.71%
Type V_4						
$\bar{x} \pm S_{\bar{x}}$	1.69 ± 0.24	0.40 ± 0.14	3.16 ± 0.37	3.16 ± 0.37	-	0.22 ± 0.68
s	0.0576	0.0196	0.1369	0.1369	-	0.4624
S%	134.12%	114.28%	73.83%	73.83%	-	27.84%
Type V_5						
$\bar{x} \pm S_{\bar{x}}$	2.21 ± 0.76	0.50 ± 0.42	3.26 ± 0.31	3.26 ± 0.31	0.10 ± 0.06	0.32 ± 0.65
s	0.5776	0.1764	0.0961	0.0961	0.0036	0.4225
S%	175.4%	142.85%	76.16%	76.16%	200%	40.50%
Type V_6						
$\bar{x} \pm S_{\bar{x}}$	2.40 ± 0.80	0.60 ± 0.71	3.77 ± 0.07	3.77 ± 0.07	0.12 ± 0.08	0.50 ± 0.28
s	0.6400	0.5041	0.0049	0.0049	0.0036	0.0784
S%	190.47%	171.43%	88.08%	88.08%	240%	63.29%

Note: $\bar{x} \pm S_{\bar{x}}$ (average ± standard deviation of the average), s (standard deviation), S% (variability coefficient)

Table 3

Comparative aspects regarding the reactivity of vitroplantlets of *Nephrolepis exaltata* Schott cultivated on modified Murashige–Skoog (1962) media (MB), with a varied content of growing regulators, where: **V_0**- MB with no growing regulators; **V_1**- MB with adding of 1 mg/l *β indolilbutiric acid* (AIB); **V_2** - MB with adding of 1.5 mg/l AIB; **V_3** - MB with adding of 2 mg/l AIB; **V_4** - MB with adding of 1 mg/l *α naftilacetic acid* (ANA); **V_5** - MB with adding of 1.5 mg/l ANA; **V_6** - MB with adding of 2 mg/l ANA, at *90 days* from inoculation.

Biometrics / Statistics evaluation	Number of roots	Roots length	Total number of leaves	Maxim dimension of leaves	Diameter of callus	Number of propagules
Type V_0						
$\bar{x} \pm S_{\bar{x}}$	4.27 ± 0.24	0.50 ± 0.10	4.61 ± 0.07	0.46 ± 0.23	-	1.18 ± 0.41
s	0.0576	0.0100	0.0049	0.0529	-	0.1681
S%	100%	100%	100%	100%	-	100%
Type V_1						
$\bar{x} \pm S_{\bar{x}}$	4.50 ± 0.14	0.30 ± 0.40	4.74 ± 0.08	0.71 ± 0.34	-	1.50 ± 0.25
s	0.0196	0.1600	0.0064	0.1156	-	0.0625
S%	105.38%	60%	102.82%	154.34%	-	127.12%
Type V_2						
$\bar{x} \pm S_{\bar{x}}$	4.57 ± 0.15	0.40 ± 0.20	5.08 ± 0.15	0.92 ± 0.44	-	1.52 ± 0.26
s	0.0225	0.0400	0.0225	0.1936	-	0.0676
S%	107.02%	80%	110.19%	200%	-	128.81%
Type V_3						
$\bar{x} \pm S_{\bar{x}}$	4.69 ± 0.16	0.45 ± 0.10	5.33 ± 0.26	1.02 ± 0.48	-	1.72 ± 0.62
s	0.0256	0.0100	0.0676	0.2304	-	0.3844
S%	109.,86%	90%	115.61%	221.74%	-	145.76%
Type V_4						
$\bar{x} \pm S_{\bar{x}}$	4.08 ± 0.22	0.50 ± 0.10	4.24 ± 0.25	0.24 ± 0.42	-	0.48 ± 0.70
s	0.0484	0.0100	0.0625	0.1764	-	0.4900
S%	95.55%	100%	91.97%	52.17%	-	40.67%
Type V_5						
$\bar{x} \pm S_{\bar{x}}$	4.50 ± 0.15	0.60 ± 0.20	4.50 ± 0.07	0.50 ± 0.32	-	0.66 ± 0.67
s	0.0225	0.0400	0.0049	0.1024	-	0.4489
S%	105.38%	120%	97.61%	108.69%	-	55.93%
Type V_6						
$\bar{x} \pm S_{\bar{x}}$	4.66 ± 0.16	0.75 ± 0.50	5.00 ± 0.16	0.60 ± 0.33	-	0.75 ± 0.65
s	0.0256	0.2500	0.0256	0.1089	-	0.4225
S%	109.13%	150%	108.46%	130.43%	-	63.56%

Note: $\bar{x} \pm S_{\bar{x}}$ (average ± standard deviation of the average), s (standard deviation), S% (variability coefficient)

CONCLUSIONS

The inocules consisting of explants type stolon apexes, prevailed from mother plants of *N. exaltata* Schott grown in a greenhouse, in a regime of *vitroculture*, on Murashige-Skoog (1962) (11) culture media, with adding if auxines (AIB and ANA), in concentrations of 1, 1.5 or 2 mg/l, they had a similar evolution, as regards *morphogenesis*, respectively *organogenesis*,

during the vitroculture, respectively for *90 days* from the initiation of experiments. Thus:

a – in comparison to the explants of feriga, which at 90 days of vitroculture registered a maximum of 5 roots/explant (whose length was of 0.45 cm), at the variant that presented in the culture media exclusively 2 mg/l AIB, the other auxin used for experiments, respectively ANA, at identical concentrations, acted in a similar way, but the process of risogenesis was inferior to that made by AIB;

b – at the level of inoculi of *N. exaltata* Schott, at 90 days from inoculation, the filogenesis lead to a forming of 5 leaves on average, with the maximum average length of 1 cm, at both studied variants;

c - *calusogenesis* was present at fitoinoculi of *N. exaltata* Schott; but, at the level of caluses there wasn't noticed any organogenesis, not until the end of experiments, at 90 days from their initiation; the phenomenon of senescence at the level of the calus appeared around the age of 60 days from the starting of vitrocultures.

REFERENCES

1. Ambrósio S. T., N. F.de Melo, 2004, Interaction between sucrose and pH during in vitro culture of *Nephrolepis biserrata* (Sw.) Schott (Pteridophyta). Acta Botanica Brasilica, 18(4), 56-59.
2. Fernández H., M. A. Revilla, 2003, In vitro culture of ornamental ferns. Plant Cell Tissue Organ Culture, 73(1), 1-13.
3. Hegdel S., V. K. Menon1, R. Noronhal, L. D'Souza, 2006, In vitro cellular & developmental biology - Plant. (eds.): Springer Berlin / Heidelberg, 42(6), 508-513.
4. Lazar A., C. Petolescu, S. Popescu, 2010, Studies concerning the in vitro multiplication of *Nephrolepis exaltata* Fluffy Ruffles. Journal of Horticulture, Forestry and Biotehnology, 14(3), 191-193.
5. Martin K.P., S. Sini, C. L. Zhang, A. P. Slater, P. V. Madhusoodanan, 2006, Efficient induction of apospory and apogamy in vitro in silver fern (*Pityrogramma calomelanos* L.). Plant Cell Report, 25(12), 1300-1307.
6. Murashige T., F. Skoog, 1962, A revised medium for rapid growth bioassays with tobacco tissue cultures. Physiologia Plantarum, 15, 473-497.
7. Pessoa C.C., A. A. L. Silva, E. T. H. Franco, D. A. Bisognin, 2004, Propagação in vitro de *Nephrolepis exaltata* Schott, Caderno de Pesquisa, Séria Biologica, Santa Cruz do Sul, 16(1), pp. 43-49.
8. Pop Maghiar R., 2012, The morphogenesis of *Nephrolepis exaltata* Schott vitocultures prevailed from stolons apexes, cultivated on aseptic media with citokinine content. Analele Universităţii din Oradea - Fascicula Protectia Mediului, 18, 436-443.
9. Somer L., R. Arbesú1, M. A. Menéndez, R. H. Fernández, 2009, Sporophyte induction studies in ferns in vitro. Euphytica, (eds.): Springer Netherlands, 171(2), 203-210
10. Torres K.C., 1989, Propagation of *Nephrolepis*. In Reinhold, V.N. (eds.): Tissue Culture Techniques for Horticultural Crops. New York, 97-102, 106-110, 181-185.
11. Zamora P. M., 2000, Urban Ferns and Fern Allies. Guide to Philippine Flora and Fauna, Quezon City, University of the Philippines Press, 58-99.