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

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Abstract

This study establishes the influence of various concentrations of citokinine on Nephrolepis exaltata Schott vitrocultures. The kinetin and benzyladenine, as growing substances, determine an intensification of phylogenesis and callus genesis at the level of explants prevailed from stolons apexes; on the other hand, risogenesis is not recorded. Concentrations of 1 mg/l, 1.5 mg/l and 2 mg/l kinetin and N^6-benzyladenine in Murashige-Skoog culture media (1962) (8) influence morphogenesis and organogenesis in a positive way at the level of Nephrolepis exaltata Schott explants.

Key words: citokinine, growing substance, *Nephrolepis exaltata* Schott.

INTRODUCTION

The stolons are crawling trunks or branches that grow roots and form a new plant in the places where they make contact to the soil. The stolon– for *Nephrolepis* – are formed out of risomes. At *Nephrolepis* the stolons are prostelic and have a protoxilem. The stolons have pluricellular scaly peri, on the margins of which there are pedunculate peri formed of small papilate cells, with sizes between 0.3-0.6 mm in height and 2.6-5 mm in length. The artificial vegetative multiplication at *Nephrolepis* can be done by cutting, using primary or secondary stolons. 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) (1-4, 9-10).

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 (5, 6). The evolution of the stolon apexes was followed for 90 days, their reaction being studied in dependence to the presence of the hormone KIN and BA 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 kinetin (KIN) and N^6-benzyladenine (BA), respectively 1, 1.5 and 2 mg/l, used in Murashige-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 then 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. 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 (9).

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 kinetin (KIN) and N^6-benzyladenine (BA) citokinines, as adding to the basic medium, in 6 variants:

V_0- Murashige-Skoog (1962) (MS) basic medium (MB) –witness lot;
V_1- MS-MB with adding of 1 mg/l kinetin (KIN);
V_2- MS-MB with 1.5 mg/l KIN;
V_3- MS-MB with 2 mg/l KIN;
V_4- MS-MB with 1 mg/l N^6-benzyladenine (BA);
V_5- MS-MB with 1.5 mg/l BA;
V_6- MS-MB with 2 mg/l BA.

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 photographed, and then were presented and discussed in the analysis part of the experimental results (Fig. 1-3).

RESULTS AND DISCUSSIONS

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

-at *30 days* from the initiation of the vitroplantlets mentioned above, *the risogenesis* was not present at any variant of culture medium that contains KIN, this phenomenon being absent up to the *90th day* from the explants inoculation (Table 1);

-at *30 days* from inoculation at all variants that contain KIN in concentrations between 1 mg/l and 2 mg/l, *the phylogenesis* was the neo-formation of a single leaf with the average length of maximum 1 cm at the level of inocules (Table 1);

- but, at *60 days,* at the experimental variants that have 1 mg/l KIN (V_1) or 1.5 mg/l KIN (V_2) in the medium, the 5 neo-leaves reached an average length of maximum 2 cm (Table 2), and at variant V_3 – with 2 mg/l KIN – the 7 leaves reached an average length of maximum 3 cm (Table 2);

- at *90 days* of vitroculture at variants that have 1 mg/l KIN (V_1) or 1.5 mg/l KIN (V_2) in the medium, the inocules neo-formed 6 leaves with the average length of maximum 2 cm (Table 3), and at variant V_3 – with 2 mg/l KIN – the size of the 7 leaves reached lengths of 4 cm (Table 3);

- the *calusogenesis,* at variants that contain KIN, was present even from the *30 days* from the initiation of experiments, this process increased in time, up to *90 days* from mounting the vitrocultures; the most prolific variant – from this point of view – was that with the biggest quantity of growing regulator, respectively 2 mg/l KIN (V_3) (Tables 1-3).

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

- the risogenesis was absent at all variants that contained BA (Tables 1-3);

- the phylogenesis was present from *30 days* of vitroculture, after this process the single leaf neo-formed at the level of explants had an average length of maximum 1 cm (Table 1), only at variants that contained 1.5 mg/l (V_5), respectively 2 mg/l benzyl-adenine (V_6);

- at *60* respectively *90 days* form the initiation of experiments, all variants that had BA citokinine in the medium at the level of explants regenerated a leaf with the average length of maximum 1 cm (Tables 2-3);

- at the explants of *N. exaltata* Schott the phenomenon of calusogenesis was present from the first *30 days* of vitroculture (Table 1), the resulting callus proliferated, at *90 days* from the initiation of cultures, leaves with the average length of maximum 1 cm.

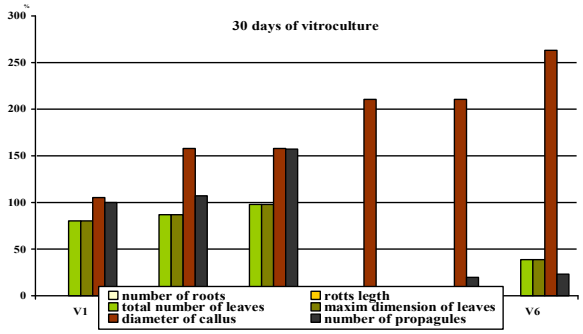

Figure 1. Biometric data regarding the growth of ***Nephrolepis exaltata*** Schott vitro-plantlets after *30 days* of vitroculture, in the following variants: $\mathbf{V_1}$- MB with 1 mg/l *kinetin* (KIN), $\mathbf{V_2}$- MB with 1.5 mg/l KIN, $\mathbf{V_3}$- MB with 2 mg/l KIN, $\mathbf{V_4}$- MB with 1 mg/l *benzyl-adenine* (BA), $\mathbf{V_5}$- MB with 1.5 mg/l BA, $\mathbf{V_6}$- MB with 2 mg/l BA, compared to the parameters recorded at the level of vitro-plantlets from the witness lot ($\mathbf{V_0}$**).**

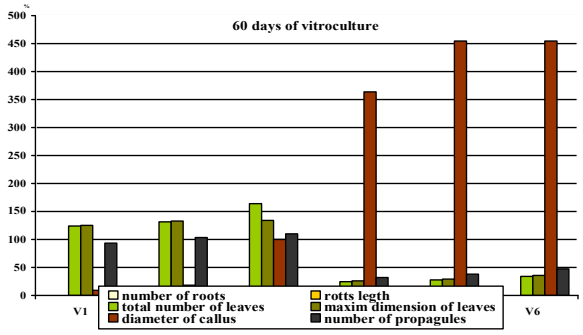

Figure 2. Biometric data regarding the growth of ***Nephrolepis exaltata*** Schott vitro-plantlets after *60 days* of vitroculture, in the following variants: $\mathbf{V_1}$- MB with 1 mg/l *kinetin* (KIN), $\mathbf{V_2}$- MB with 1.5 mg/l KIN, $\mathbf{V_3}$- MB with 2 mg/l KIN, $\mathbf{V_4}$- MB with 1 mg/l *benzyladenine* (BA), $\mathbf{V_5}$- MB with 1.5 mg/l BA, $\mathbf{V_6}$- MB with 2 mg/l BA, compared to the parameters recorded at the level of vitro-plantlets from the witness lot ($\mathbf{V_0}$).

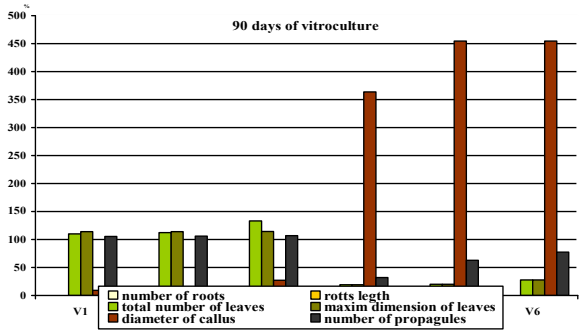

Figure 3. Biometric data regarding the growth of ***Nephrolepis exaltata*** Schott vitro-plantlets after *90 days* of vitroculture, in the following variants: $\mathbf{V_1}$- MB with 1 mg/l *kinetin* (KIN), $\mathbf{V_2}$- MB with 1.5 mg/l KIN, $\mathbf{V_3}$- MB with 2 mg/l KIN, $\mathbf{V_4}$- MB with 1 mg/l *benzyladenine* (BA), $\mathbf{V_5}$- MB with 1.5 mg/l BA, $\mathbf{V_6}$- MB with 2 mg/l BA, compared to the parameters recorded at the level of vitro-plantlets from the witness lot ($\mathbf{V_0}$**).**

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 *kinetin* (KIN); **V_2** - MB with adding of 1.5 mg/l KIN; **V_3** - MB with adding of 2 mg/l KIN; **V_4** - MB with adding of 1 mg/l *benzyladenine* (BA); **V_5** - MB with adding of 1.5 mg/l BA; **V_6** - MB with adding of 2 mg/l BA; 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						
$\bar{x} \pm S_{\bar{x}}$	0.09 ± 0.15	0.25 ± 0.12	1.37 ± 0.31	1.37 ± 0.31	0.19 ± 0.19	0.56 ± 0.10
s	0.0225	0.0144	0.0961	0.0961	0.0361	0.0100
S%	100%	100%	100%	100%	100%	100%
Type V_1						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.10 ± 0.19	1.10 ± 0.19	0.20 ± 0.25	0.56 ± 0.10
s	-	-	0.0361	0.0361	0.0625	0.0100
S%	-	-	80.29%	80.29%	105.26%	100%
Type V_2						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.19 ± 0.13	1.19 ± 0.13	0.30 ± 0.32	0.60 ± 0.11
s	-	-	0.0169	0.0169	0.1024	0.0121
S%	-	-	86.86%	86.86%	157.89%	107.14%
Type V_3						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.34 ± 0.32	1.34 ± 0.32	0.30 ± 0.32	0.88 ± 0.15
s	-	-	0.1024	0.1024	0.1024	0.0225
S%	-	-	97.81%	97.81%	157.89%	157.14%
Type V_4						
$\bar{x} \pm S_{\bar{x}}$	-	-	-	-	0.40 ± 0.41	-
s	-	-	-	-	0.1681	-
S%	-	-	0%	0%	210.52%	0%
Type V_5						
$\bar{x} \pm S_{\bar{x}}$	-	-	0.11 ± 0.91	0.11 ± 0.91	0.40 ± 0.41	0.11 ± 0.85
s	-	-	0.8281	0.8281	0.1681	0.7225
S%	-	-	8.02%	8.02%	210.52%	19.64%
Type V_6						
$\bar{x} \pm S_{\bar{x}}$	-	-	0.53 ± 0,72	0.53 ± 0.72	0.50 ± 0.56	0.13 ± 0.81
s	-	-	0.5184	0.5184	0.3136	0.6561
S%	-	-	38.68%	38.68%	263.15%	23.21%

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: $\mathbf{V_0}$- MB with no growing regulators, $\mathbf{V_1}$- MB with adding of 1 mg/l *kinetin* (KIN), $\mathbf{V_2}$- MB with adding of 1.5 mg/l KIN, $\mathbf{V_3}$- MB with adding of 2 mg/l KIN, $\mathbf{V_4}$- MB with adding of 1 mg/l *benzyladenine* (BA), $\mathbf{V_5}$- MB with adding of 1.5 mg/l BA, $\mathbf{V_6}$- MB with adding of 2 mg/l BA, 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	4.26 ± 0.16	4.06 ± 0.33	0.11 ± 0.16	1.69 ± 0.35
s	0.0225	0.0400	0.0256	0.1089	0.0256	0.1225
S%	100%	100%	100%	100%	100%	100%
Type V_1						
$\bar{x} \pm S_{\bar{x}}$	-	-	5.28 ± 0.19	5.08 ± 0.09	0.01 ± 0.01	1.58 ± 0.22
s	-	-	0.0361	0.0081	0.0001	0.0484
S%	-	-	123.94%	125.12%	9.09%	93.49%
Type V_2						
$\bar{x} \pm S_{\bar{x}}$	-	-	5.60 ± 0.24	5.39 ± 0.11	0.02 ± 0.03	1.75 ± 0.04
s	-	-	0.0576	0.0121	0.0009	0.0016
S%	-	-	131.45%	132.75%	18.18%	103.55%
Type V_3						
$\bar{x} \pm S_{\bar{x}}$	-	-	6.99 ± 0.35	5.44 ± 0.12	0.11 ± 0.16	1.86 ± 0.08
s	-	-	0.1225	0.0144	0.0256	0.0064
S%	-	-	164.08%	133.99%	100%	110.05%
Type V_4						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.05 ± 0.82	1.05 ± 0.82	0.40 ± 0.41	0.54 ± 0.81
s	-	-	0.6724	0.6724	0.1681	0.6561
S%	-	-	24.64%	25.86%	363.63%	31.95%
Type V_5						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.18 ± 0.71	1.18 ± 0.71	0.50 ± 0.56	0.64 ± 0.67
s	-	-	0.5041	0.5041	0.3136	0.4489
S%	-	-	27.69%	29.06%	454.54%	37.86%
Type V_6						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.45 ± 0.65	1.45 ± 0.65	0.50 ± 0.56	0.80 ± 0.61
s	-	-	0.4225	0.4225	0.3136	0.3721
S%	-	-	34.03%	35.71%	454.54%	47.33%

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: $\mathbf{V_0}$- MB with no growing regulators, $\mathbf{V_1}$- MB with adding of 1 mg/l *kinetin* (KIN), $\mathbf{V_2}$- MB with adding of 1.5 mg/l KIN, $\mathbf{V_3}$- MB with adding of 2 mg/l KIN, $\mathbf{V_4}$- MB with adding of 1 mg/l *benzyladenine* (BA), $\mathbf{V_5}$- MB with adding of 1.5 mg/l BA, $\mathbf{V_6}$- MB with adding of 2 mg/l BA, 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						
$\bar{x} \pm S_{\bar{x}}$	2.27 ± 0.24	0.50 ± 0.10	5.57 ± 0.07	5.05 ± 0.40	0.11 ± 0.16	2.18 ± 0.05
s	0.0576	0.0100	0.0049	0.1600	0.0256	0.0025
S%	100%	100%	100%	100%	100%	100%
Type V_1						
$\bar{x} \pm S_{\bar{x}}$	-	-	6.13 ± 0.02	5.75 ± 0.14	0.01 ± 0.01	2.30 ± 0.08
s	-	-	0.0004	0.0196	0.0001	0.0064
S%	-	-	110.05%	113.86%	9.09%	105.50%
Type V_2						
$\bar{x} \pm S_{\bar{x}}$	-	-	6.25 ± 0.05	5.76 ± 0.14	0.01 ± 0.01	2.31 ± 0.08
s	-	-	0.0025	0.0196	0.0001	0.0064
S%	-	-	112.20%	114.05%	9.09%	105.96%
Type V_3						
$\bar{x} \pm S_{\bar{x}}$	-	-	7.42 ± 0.09	5.77 ± 0.14	0.03 ± 0.05	2.33 ± 0.09
s	-	-	0.0081	0.0196	0.0025	0.0081
S%	-	-	133.21%	114.25%	27.27%	106.88%
Type V_4						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.07 ± 0.88	1.07 ± 0.88	0.40 ± 0.41	0.70 ± 0.80
s	-	-	0.7744	0.7744	0.1681	0.6400
S%	-	-	19.21%	19.21%	363.63%	32.11%
Type V_5						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.12 ± 0.80	1.12 ± 0.80	0.50 ± 0.56	1.37 ± 0.61
s	-	-	0.6400	0.6400	0.3136	0.3721
S%	-	-	20.10%	20.10%	454.54%	62.84%
Type V_6						
$\bar{x} \pm S_{\bar{x}}$	-	-	1.55 ± 0.66	1.55 ± 0.66	0.50 ± 0.56	1.69 ± 0.52
s	-	-	0.4356	0.4356	0.3136	0.2704
S%	-	-	27.82%	27.82%	454.54%	77.52%

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 stolons apexes, prevailed from mother plants of *N. exaltata* Schott grown in a greenhouse, in a regime of *vitroculture*, on Murashige-Skoog (1962) (10) culture media, with adding if citokinines (KIN and BA), 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 the presence of KIN or BA citokinines, in different concentrations in the culture medium, *risogenesis* was absent at the species I experimented on;

b – at *N. exaltata fern*, the *phylogenesis* – at *90 days* from the explants inoculation on aseptic media with a content of KIN, in a quantity of 2 mg/l – consisted in the regeneration, in the apical area, of an average of 7 leaves, with an average length of maximum 4 cm;

c – at explants of *N. exaltata* Schott *calusogenesis* was present from the first *30 days* from inoculation, process that increased until the end of the experiments; but, at the examinations performed in the 90th day of vitroculture, at the level of the explants cultivated on the variants with KIN in the medium, at the level of the callus there could be noticed a process of senescence, even if in the apical area of phyto-inocules the callus had about 7 neo-formed leaves with a maximum size of 4 cm.

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