THE ROLE OF SOME ALKYL CHEMICAL AGENTS IN THE *IN VITRO* MORPHOGENESIS OF SOME SPECIES AND THEIR INVOLVEMENT IN IMPROVEMENT AND ECOLOGY

ROLUL UNOR AGENȚI CHIMICI ALCHILANȚI ÎN MORFOGENEZA *IN VITRO* A UNOR SPECII ŞI IMPLICAȚIILE LOR ÎN AMELIORARE ŞI ECOLOGIE

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Abstract: *The paper deals with the in vitro behaviour of some explants (apex, node), prevailed from the esparcet species Onobrichis vicifolia Scop., for the regeneration of species, their multiplication and application of a mutagen (table 1). The apex regenerated plants completely formed with multiplication V_2 and V_3 (media that proved to be the best). The node also generated neo plantlets completely confirmed with a high regenerative capacity on almost all variants. On media with Zeatin the regeneration percentage reached 95 – 99%. The media with 2,4 D produced a callus from both explants, the biggest mass of embryogen callus, with visible embryos and a regenerative capacity were obtained from apex V_7 and V_9. The embryogen callus treated with mutagen medium in low concentration showed a good regenerative capacity especially on media with dimethylsulphonate (DMS).*

Rezumat: *Utilizarea unor substanțe chimice cu capacitate de a induce mutageneza in vitro are mari perspective în ameliorare. Culturile in vitro au rol esențial în programele de ameliorare a plantelor, permițând selecția şi multiplicarea unor linii productive, rezistente la stres climatic, boli şi dăunători. În cercetările noastre anterioare au fost testate la câteva specii cultivate in vitro, cele doua substanțe chimice alchilante (dietilsulfonat – DES şi dimetilsulfonat-DMS), cu rezultate remarcabile în direcția inducerii mutagenezei.*

Key words**:** *in vitro morphogenesis, esparcet, Onobrichis vicifolia Scop., in vitro regeneration, apex, node, diethyl sulphonate (DES), dimethylsulphonate (DMS)*
Cuvinte cheie: *morfogeneză in vitro, sparcetă, Onobrichis vicifolia Scop., regenerare in vitro, apex, nod, dietilsulfonat, dimetilsulfonat*

INTRODUCTION

The programs of ameliorating the evergreen forage leguminous plants allow using *in vitro* cultures for the selection and multiplication of some productive lines, resistant to stress (5). For some of these species we obtained remarkable results as regards the testing of the *in vitro* reaction capacity, in order to raise the clonal multiplication ratio, to generate embryogen callus and to induce mutagenesis (6, 7, and 8). *In vitro* culture has the aim to preserve some new created lines or species, to observe their *in vitro* reaction, as regenerative capacity, multiplication, obtaining embryogen callus from various explants, depending on the hormonal balance from the culture medium (3).

Using some chemical substances with a capacity to induce *in vitro* mutagenesis also has a great perspective in amelioration. *In vitro* cultures have an essential role in the programs of plants amelioration, allowing the selection and multiplication of a productive line, resistant to climatic stress, diseases and pests. In the previous research two alkyl chemical substances (diethyl sulphonate – DES and dimethylsulphonate - DMS) were tested for some species of

leguminous plants, with remarkable results in inducing the mutagenesis (2, 7, 8). The paper has an objective to study the influence of DES and DMS on the evolution of plants at *Onobrichis vicifolia* Scop. obtained *in vitro* and to observe their mutagen effect on the tissues detached from the species.

MATERIALS AND METHOD

The seeds of esparcet were sterilised with the well known classical technique (1) and inoculated on a MS base medium, with half macro elements and microelements. After about 10 days from inoculation, the seeds germinated and in other 15 days they formed completely conformed plantlets. From these explants (*apex* of about 3 mm and *node)*, tissues that were re-inoculated on media with EDS and DMS content, with two times (24 and 48 hours) of treatment (table 1). The media with content of mutagen chemical agents were abbreviated with M_o to M_8, the basic medium being MS -1962(4).

Table 1

Medium with DES and DMS content and with different times of treatment

Variant	Basic media	DES conc.	Treatment time (hours)	Variant	DMS conc.	Treatment time (hours)
M_0	MS	Control - witness				
M_1	MS	4 ppm.	24 h	M_5	4 ppm.	24 h
M_2	MS	3 ppm.	24 h	M_6	3 ppm.	24 h
M_3	MS	2 ppm.	48 h	M_7	2 ppm.	48 h
M_4	MS	1 ppm.	24 h	M_8	1 ppm	24 h

MS = Murashige – Skoog, 1962; DES = diethylsulphonate; DMS = dimetylsulphonate

After the treatment time, the explants are re-inoculated on new culture media with a content of hormonal substances and it was observed the induction of callus genesis, of organogenesis, *in vitro* morphogenesis and callus production. The media experimented in this respect are shown in table 2 (abbreviated with V_o to V_9).

Table 2

Composition of the culture media with a content of growing hormones and 2,4D

Variant	Basic media	Composition Z	hormonal BA	mg/l AIA	AIB	2,4D
V_0	MS 1/2	-	-	-	-	-
V_1	MSC 1/2	-	-	-	-	-
V_2	MS	1.0	-	-	0.5	-
V_3	MS	2.0	-	0.5		-
V_4	MS	-	2.0	-	0.5	-
V_5	MS	-	4.0	0.5	-	-
V_6	MS	1.0				2.0
V_7	MS	0.5				4.0
V_8	MS	-	2.0	-	-	2.0
V_9	MS	-	1.0	-	-	4.0

MS = Murasige- Skoog-1962; MS1/2 = half with macro and micro;
MSC1/2 = cu 5 g/l vegetal coal; Z = zeatin; BA = benzyl adenine;
AIB = indolil butyric acid; AIA = indolil acetic acid; 2,4D = DTT

RESULTS AND DISCUSSION

After about six weeks of re-cultivation on the media shown in table 2, we observed some macroscopic parameters of plants' developing and growth: the evolution of the explants as regards the regeneration percentage, the number of regenerated plants, the conformation of esparcet neo-plantlets regenerated *in vitro*, the presence of the root system. It was also observed the callus forming on media with 2,4D (colour, nature, structure, weight and diameter of the callus mass), the protein content of esparcet regenerated callus, the mutagen effect of DES and DMS on the callus.

In the present paper we present two aspects of our experiment (which is more vast), that is: the *in vitro* regenerative capacity of the esparcet node and apex, depending on the hormonal balance (table 3); and obtaining callus *in vitro* (table 4) and also some aspects

regarding the effect of DES and DMS on some tissues obtained *in vitro* (on neo-plantlets or callus).

In vitro behaviour aspects of esparcet apex

The obtained data show that this tissue presents a good regenerative capacity. On media without growth hormones (V_0, V_1) only a plantlet regenerates, with a height of about 3.8 – 4.5, but the roots system is weak and the regenerative capacity only over 50%. The regenerative capacity exceeds 80% on media with Z and even 90% on V_3, variant with the greatest number of plantlets. The plantlets obtained on V_4 are in a smaller number but they are completely conformed and also the root system especially in the presence of AIB, auxine known as being strongly involved in risogenesis (6). On media with BA, the apex has also a remarkable regenerative capacity but inferior to the media with Z (see table 3).

It has to be mentioned the fact that mutagen substances did not determined visible modifications, macroscopic at the neo-plantlets obtained *in vitro* from the apex. The diameter of callus mass reached the maximum value of 1.8 cm on medium V_7 (with 0.5 ppm – Z + 4 ppm 2,4D). The callus obtained from the apex treated with 4 ppm and 3 ppm DES, for 48 hours gave birth to a callus slightly friable, yellow green, without embryos, the substance inhibiting the harmonious evolution of esparcet culture and even the protein synthesis (the latter aspect will be presented in another paper). But a lower concentration of mutagen substances (DMS) 1 ppm has a favourable effect on the culture and the protein content in the callus.

In vitro behaviour of esparcet node

Looking at table 3 we can notice that the node has a high regenerative capacity even on media without hormones. For this explant on Zeatin media it was obtained the greatest number of regenerated plants, at a concentration of 2 ppm with a regeneration percentage of almost 100%. The root system was stimulated by the presence of AIB – 0.5 ppm. On media with 2,4D the node generated callus in a percentage of 70-82%, light green and soft, on media with Zeatin and dark green on media with benzyl adenine. The callus mass reached the maximum diameter on V_7 (Z – 0.5 ppm + 2,4D – 4 ppm). On media with alkyl chemical substances, the node behaved similar to the apex.

Table 3

In vitro regenerative capacity of esparcet node and apex cultivated on media with auxine AND cytokinine ($V_o - V_6$)

Var.	Explant	No. Of regenerated neo plantlets	Height of neo plantlets (cm)	No. of roots	Length of root (cm)	Regeneration capacity %
V_o	apex	1	3.8	1	0.4	50
V_1		1	4.5	2	0.2	59
V_2		3	2.4	7	1.2	87
V_3		5	2.2	2	0.6	90
V_4		3	2.8	4	0.4	80
V_5		3	2.0	5	0.8	80
V_o	node	2	3.4	1	0.3	61
V_1		2	4.4	1	0.2	65
V_2		4	2.3	8	0.9	95
V_3		6	2.0	4	0.5	99
V_4		3	2.0	8	1.5	75
V_5		4	1.8	3	0.4	78

CONCLUSIONS

1. The esparcet *apex* on Zeatin and AIB media generates the greatest number of neo plantlets with the best root system. On the medium with benzyl adenine (BA) the regeneration and multiplication of apex is good but inferior to zeatin.
2. At neo plantlets obtained from apex there wasn't any modification caused by the treatment with DES or DMS.
3. But the callus obtained from the *apex* on the variants treated with DES and DMS in a high concentration (3 ppm and 4 ppm/48h) generate plantlets that don't grow harmoniously. At a

low concentration of DES and DMS (1 ppm/24h), the callus generates plantlets normally developed, with a high number of proteins.

4. The reaction of the *node* cultivated *in vitro* is superior to the apex, even in media without hormones (V_o, V_1) where the regeneration percentage is only 65%.

Table 4

Generating callus and its evolution on media with 2,4D and hormones, depending the nature of the tissue

Variant	Explant	Diameter of callus „cm"	Colour	Consistency	Regeneration capacity of callus (%)
V_o	apex	-	-	-	-
V_1		-	-	-	-
V_6		1.0	olive green	soft	88
V_7		1.8	olive green	Semi-soft with embryos	95
V_8		1.0	green	Slightly soft	78
V_9		1.2	yellow green	Semi-soft with embryos	72
V_o	node	-	-	-	-
V_1		-	-	-	-
V_6		0.4	white green	Soft	70
V_7		1.0	olive green	Soft	82
V_8		0.4	dark green	Hard	70
V_9		0.8	dark green	Hard	72

5. The regeneration percentage of the node on media with zeatin – 2 ppm reaches 99%, and the root system in the presence of AIB reaches about 8 roots of 0.9-1.5 cm.

6. The benzyl adenine in moderate doses (2 ppm) has a stimulating effect, but inferior to Zeatin. The indolil-butyric acid (AIB) proves to be a good stimulator for producing the root system.

7. The dimethylsulphonate in a concentration of 2 ppm/48h (V_7), determines the decrease of the protein content in the tissue, while the low concentration of DMS - 1ppm/24h (V_8) determines an increase of the protein content following the analysis of the callus.

8. This type of research is of great interest for the papers concerning the amelioration of culture plants, but it can also be of ecologic interest.

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