THE INFLUENCE OF FLUOROGLUCINOL ON THE *IN VITRO* CULTURE AT ESPARCET (*ONOBRYCHIS VICIIFOLIA* SCOP)

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Abstract

Used in order to regenerate and multiply the plant tissues in vitro, the fluoroglucinol proved efficient to some species which react with difficulty at in vitro culture (strelizia, musa, magnolia etc.). The species taken under study, Onobrychis viciifolia Scop., has the best in vitro reaction regarding the regeneration and multiplication, in comparison to other fodder vegetables. In this study the mersitem, apex were studied (detached from plants resulted from the germination of seeds in vitro), on averages with fluoroglucinol added in MS environment in dosage of 100 mg/l, either alone, or in mixture with phytohormones. The efficiency of fluoroglucinol is manifested in mixture with 2iP- 1,0 mg/l, on all parameters, leading to a multiplication rate of 100%, generating the highest number of plants, with a radicular system adequate as number and length. The fluoroglucinol alone in the environment has a satisfactory efficiency, but in mixture with cytokinine (especially 2iP) its effect is maximum on all parameters (S_5). On the environments Ph free, but with BA or 2iP and in the presence AIB a callus is presented at the basis of new plants formed. The diferentiation of nodes on the radicular system formed at esparcet, we may notice, also in the absence of fluoroglucinol, on the averages 1mg/l BA, or 2iP + 0,5 mg/l AIB (S_2, S_4), approximately 2 nodes/explant of 2 -5 mm diameter. The use of concentration of 100 mg/l Ph. In addition with cytokinine (preferably 2iP), it is recommended to advantageously multiply the meristem of esparcet in vitro.

Key words: *Onobrychis viciifolia* Scop. fluoroglucinol, meristem, multiplication rate, nodes, regeneration.

INTRODUCTION

The *in vitro* culture is successfully used in the perennial fodder plant improvement programs, making possible to select and multiply valuable lines (*Varga* et al., 1998). The factors controlling *in vitro* regenerative capacity of culture plants are less known; in case of fodder vegetables, priority factors involved in regeneration are considered the chemical structure of substances, quantity and quality of phyto-hormones and their nature (*Phillips and Collins*, 1984). In our previous research related to fodder vegetables, the *in vitro* capacity of various explants taken from red clover, white clover, bird's foot trefoil, lucerne was studied (*Zăpârţan* et al, 1990). The increase of cloning multiplication rate, generation of callus and its sub-cultivation in order to induce the formation of new plants were followed (*Savatti* et al. 2006), as well as obtaining variability by mutagenesis *in vitro*(*Zăpârţan* et al., 2006).

Successfully used in the culture environments, the fluoroglucinol proved efficient in stimulation of growth and development of tissues, in relation to the nature of species and purpose intended (*Agud*, 2010), with results depending on the concentration of substance, and the type of explant (*Vicaş,* 2009). The substance's efficiency was demonstrated in regeneration of reticent species of plants, or even without reaction *in vitro,* such as: *Strelizia reginae* (*Ziv and Hakevy.,* 1982; *Kulcarni* at al. 2007), at which the addition of

fluoroglucinol in the culture environment proved efficient in concentration of 100mg/l, concentration which stimulated the regeneration and multiplication *in vitro* also to other species of Lilium (*Zăpârţan and Deliu*, 1995), with superior results in combination to cytokinine. If the fluoroglucinol is alone in the environment, the regeneration capacity of the tissue is inferior in comparison to the combination with a dosage of cytokinine (*Zăpârţan,* 2001) some researches signalling remarkable results to the combination Ph. with benzyl adenine (*Zăpârţan et* al. 1999 – 2000).
This work follows the effect of fluoroglucinol *in vitro* to the esparcet meristem, in relation to the presence of other phytohormones, in order to establish the multiplication rate and the regeneration capacity of the species. The less studied species from the point of view of regeneration capacity *in vitro*, using more types of explants, of different ages, with good results in relation to the composition of hormonal balance, were used (*Savatti* et al. 1991).

MATERIAL AND METHODS

In majority of experiences at the vegetables cultivated *in vitro* the study was initiated from explants prelevated from field plants, haing in general a high percentage of lossess due to infections appeared during incubation. In this experience, we started from esparcet seeds, which, after sterilization in sodium hypochlorite 20 minutes + 2-3 drops of tween were left for germination on an environment following T. *Murashige and Skoog***,** A 1962, with half of microelements and macroelements (MS1/2). After approximately 12 days from the genrmination, the following percentage took place: 72% germinated seeds, 8% stationary and 20% necrosis (Image 1). The necrosis may be caused by the disinfection treatment, or the concentration of substance or the duration of treatment.

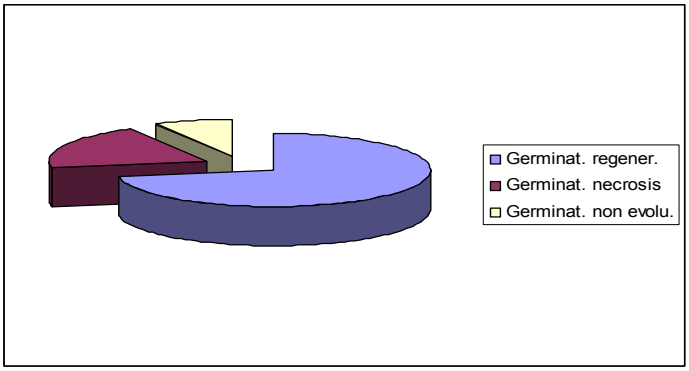

Image 1 Germination percentage in vitro of esparcet seeds
(After approximately 2 weeks)

The fluoroglucinol is experimented at esparcet on the environment following Murashige – Skoog (MS)- 1972 (*T. Murashige and Skoog***,** *A* 1962), with the following composition: macroelements, microelements and FeEDTA – MS; meso-inositol – 100mg/l; tiamine HCl, pyridoxine HCl and nicotinic acid, each 1 mg/l; saccharose – 30 g/l; agar – 7 g/l; pH=6,1, considered basic environment (MS), to which hormones were added and fluoroglucinol following the versions S_o - S_5, presented in Table 1.

Table1

The composition of environments for the culture of esparcet meristem

Option	Basic environment	Vegetal coal(g/l)	Ph. mg/l	BA mg/l	2iP mg/l	AIB mg/l	Bonification
S_o	MS1/2	3	-	-	-	-	xx
S_1	MS	-	100				xx
S_2	MS	-	-	1.0	-	0.5	xxxx
S_3	MS	-	100	1.0	-	0.5	xxxx
S_4	MS	-	-	-	1.0	0.5	xxxxx
S_5	MS	-	100	-	1.0	0.5	xxxxx

*(xx = satisfactory evolution; xxxx = good evolution; xxxxx = very good evolution)

The fluoroglucinolul in a single combination was experimented, (100mg/l) either alone, or in combination with cytokinine, in this case BA and 2iP and an auxin, AIB. 100 explants were innoculated, which, following the innoculation, all the vegetation period were maintained in the conditions of grouwth chamber, at a temperature of 20 – 24^{o}C, a photoperiod of 16 hours out of 24 and at a luminous intensity of 2.000 lux.

RESULTS AND DISCUSSION

The data emphasize that the esparcet manifests a good regenerative and multiplication capacity *in vitro*, influenced by the presence of fluoroglucinol, but also of other growth phytohormones, especially cytokinines. The results of observations being signalled in Table 2, at over three months of culture *in vitro*. The chart contains the average of parameters analyzed: regeneration percentage, neoformation of plants and radicular system, height and length, multiplication rate.

The neoformation of plantlets and multiplication *in vitro* of the species *Onobrychis viciifolia* Scop., on environments with fluoroglucinol emphasizes a good percentage of regeneration, of 60 and 99%, determined by the association in the environment of fluoroglucinol with a cytokinine and an auxine. Image 2 emphasizes the effect of acid 2 isopentil adenine on the regeneration, this reaching 99%.

Table 2

Evolution of meristem of *Onobrychis viciifolia* Scop.,
(After 120 days)

Option	% Regeneration	No. pl.	Heights pl.(cm)	No. roots	Length of root (cm)	Multiplication rate	Observations
S_o	60	2	10	2	1.5	25	- 1-2 plants with poor radicular system
S_1	62	3	6	2	2.2	35	- small number of roots
S_2	80	4	3	**10**	4.0	45	-good radicular system; with 2-3 nodes
S_3	85	**8**	4	3	**6.4**	**84**	-generates callus; inhibits the roots
S_4	**90**	6	3	8	5.5	62	-good radicular system, thick roots with approximately 2 nodes
S_5	**99**	**10**	4	3	**8.0**	**100**	-generates callus; reduced number of roots.

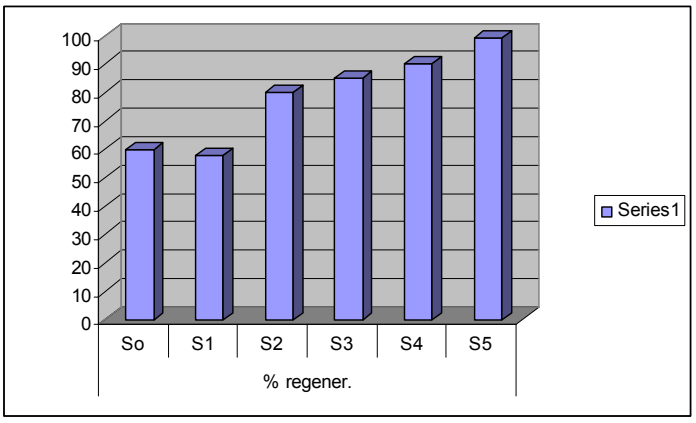

Image 2. Regeneration percentage of esparcet meristem
(After 120 days)

After 120 days of *in vitro* culture, the formation of new plants and their height is represented in a relevant manner in image 3. If the witness reaches 1-2 high plantlets, the environment only with fluoroglucinol (S_1) reaches a better average and a balanced length of esparcet neoplantlets formed *in vitro*. In this case is also emphasized the combination with cytokinine, 2iP + AIB + fluoroglucinol(S_5), on which approximately 10 esparcet plantlets of approximately 4,5 cm are formed, and at the basis a green-olive callus with friable properties. But also in the presence of benzyl adenine, the results are superior (S_3), resulting approximately 8 neoplantlets with a length of 4 cm and a callus mass at the basis of plantlets, callus with the same structure.

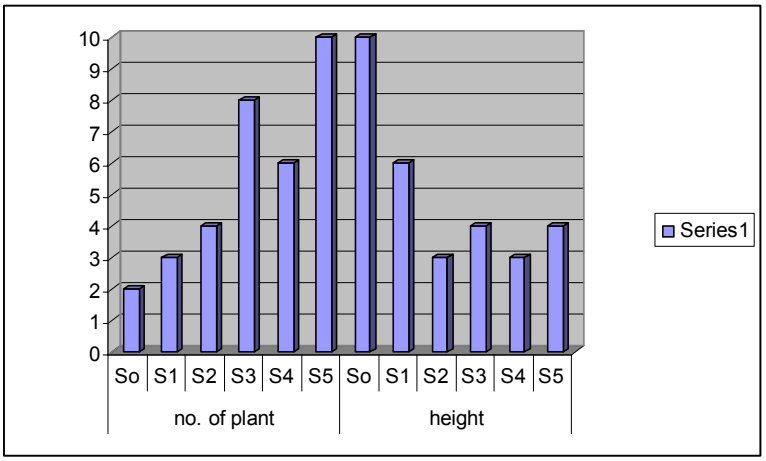

Image 3 Neoformation of esparcet plants and their heights
(After 120 days)

The radicular system, in comparison to other esparcet experiences (please consult *Savatte* 1991), is differentiated in the same period of 120 days, the highest number being obtained on the averages without fluoroglucinol (S_2 and S_4), approximately 8 – 10 roots/explant of 4-5 cm length, with 2-3 nodes/root (Image 4). The presence of roots is signalled also on the environments with fluoroglucinol and phytohormones (S_3 and S_5), in a much smaller number.

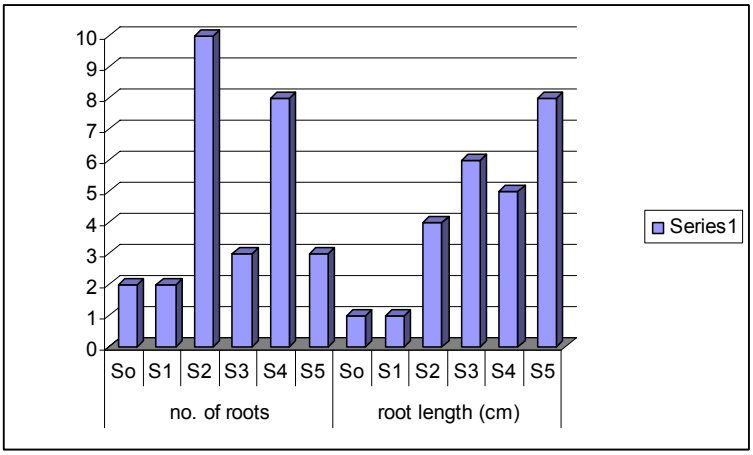

Image 4. The evolution of radicular system at the esparcet meristem
(After 120 days)

The multiplication rate of esparcet *in vitro* (diagram 5), follows the course of regeneration percentage and, in this case, the combination between cytokinine, auxine and fluoroglucinol has the best effect, reaching a rate of 100% on S_5 (1 mg/l 2iP + 0,5 mg/l AIB + 100mg/l Ph) and 85% on the environment S_3 (1mg/l BA + 0,5 mg/l AIB + 100mg/l Ph.)

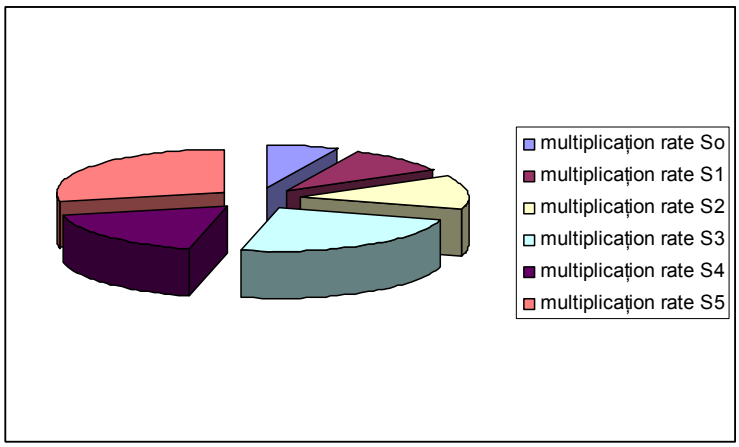

Image 5 Multiplication rate of the esparcet meristem cultivated on environments with and without fluoroglucinol

The callus generated from esparcet explants represents a valuable material in inducting the appearance of mutations or in transformation works in embryogene callus and, finally, the regeneration of plants from the same tissue. The esparcet plantlets obtained *in vitro* either with a rich radicular system, or with a poor radicular system, in approximately 40 days from plantation in open air, forms a rich radicular system, a compact bush characteristic to the species, being acclimatized in a percentage of 80%, percentage depending on the climate conditions of the period.

CONCLUSIONS

1. The fluoroglucinol may be successfully used in the culture environment for the multiplication *in vitro* of the species *Onobrychis viciifolia* Scop., either in mixture with phyto-hormones.
2. The concentration of 100 mg/l Ph. in combination with 2iP and AIB (each 1 mg/l, respectively 0, 5 mg/l) stimulates the regeneration *in vitro* in percentage of 99%.
3. The regenerated plants on options with Ph. (S_3 and S_5) and in large number and completely organized, the radicular system being inferior to the one from the versions with addition of cytokinine and auxine, but generates callus with superior structure.
4. The radicular system is formed on the environments only with cytokinine and auxine (S_2 and S_4), environments for which a friable callus mass is also formed, green – olive and approximately 2-3 nodes/roots.
5. The fluorglucinol alone, we may say that it has the same effect as vegetal coal, the evolution of witness evidence (S_o) being similar to the version S_1, with the content only of fluoroglucinol
6. We recommend, however, the fluoroglucinol in the culture environment of the species *Onobrychis viciifolia* Scop., in mixture with a cytokinine and auxine, in order to obtain a superio regeneration percent and a superior multiplication rate.

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