THE EFFECT OF THE PHLOROGLUCINOL OVER THE EXPLANTS OF RED CLOVER CULTIVATED IN VITRO (*TRIFOLIUM PRETENSE L.)*

Vicaş Gabriela*, Maria Zăpârţan*

**University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea; Romania, e-mail: gabrielavicas@yahoo.com*

Abstract

Little used with the purpose of regenerating and reproducing of tissues of plants in vitro, the phloroglucinol proved to be very efficient at some species that have a difficult reaction to the in vitro culture (strelizia, musa, magnolia, etc). In the case of the specie studied by us, Trifolium pratense L, the phloroglucinol added in the medium MS in dose of 100mls/l, it stimulated the greatest percentage of regeneration of the apex, the formation of the greatest number of neoplantules with the biggest number of afferent roots. The difference of nodes on the radicular system formed in vitro can be remarked in the environments with 100 g/l Ph + 1mg/l BA + 0,5 mg/l(V_3, V_5), about 3-5 nodes /explant with the diameter of 2-5 mms. The formation of the node takes place also in an medium formed by phloroglucinol (V_1 şi V_2), but it only formes a single node with the diameter of 1 mm. For a favorable multiplication of the species of plants in vitro, we recommend the use of a concentration of a 100 mg/l Ph. in addition to cytokinins.

Keywords: red clover, phloroglucinol, neoplantules, apex, *in vitro*

INTRODUCTION

Growth regulator from the 1,3,5 – trihydroxybenzene [$C_8H_3(OH)_3$ $2H_20$] group, the phloroglucinol can be successfully used in the medium of culture for the stimulation of growth and development of the tissues of some species of plants, with results which depend on the concentration of the substance, of the nature of the specie and of the explant. Used into an medium of culture it proved its efficiency in the regeneration of some species of reluctant plants, or even with no reaction in the aseptic medium for life. One of these species is also *Strelizia reginae,* to which, the growth of axial bud into an medium with phloroglucinol, gave excellent results in terms of the regeneration and of the multiplication in vitro. The same source specifies that at Musa spp., difficult to regenerate in vitro (from the same botanical family as Strelizia), the addition of phloroglucinol in culture medium has proved to be efficient in concentration of a 100 mg/l. The concentration of 100mg/l has stimulated the regeneration and the multiplication in vitro of some species of Lilium, with superior results in combination to a cytokinins (BA - in this case). If the phloroglucinol is single in the medium, the regeneration capacity of the tissues is inferior, in comparison with the combination with one dose of cytokinins The present study is the first part of some extensive research related to the implications of the phloroglucinol at the culture of some species of plants, which is going to be followed by other two, one concerning the effect of this substance in some flower species, and the other with the purpose of the tuberization and the bulbification *in vitro* of some varieties.

MATERIAL AND METHOD

The behavior and the capacity of regeneration in vitro of the *Trifolium pratense L* apex, in medium with phloroglucinol, is tested on an basic medium after *Murashige* –

Skoog (MS)- 1972, with the following composition: macro elements, microelements and FeEDTA – MS; myo-inosytol – 100 mg/l; thiamine HCl and nicotinic acid 1 mg/l; sucrose – 30 g/l; agar – 7 g/l; pH = 6,1, at this medium considered basic medium (MS), we also added other simulative substances, the versions being specified in Table 1. The phloroglucinol was experimented in two concentrations: 100 and 200 mgs/l, either single (V_1 and V_2), or combined with BA and 2iP – 1 mg/l + 0, 5 mg/l AIB (V_3 and V_4) and with BA and 2iP - 2 mg/l + 0,5 AIB(V_5 and V_6). For comparison we used a control version formed only by the basic medium (V_O). The *Trifolium pratense* specie was a lot studied from the point of view of its in vitro behavior and of the process of induction of mutations, with remarkable results, proving to be specie with of regeneration and multiplication in vitro, depending on the hormonal balance from the medium and of the used dose of phytohormones. The experiment was initiated in a time of year unfavorable for the development of the clover both *in vitro*, and *in vivo*, due to which we resorted to increasing the dose of phloroglucinol and cytokinins in a beneficial way.

Table 1

The culture medium with phloroglucinol, used to the *in vitro* culture of the Trifolium *pratense* L specie

No. crt.	Var.	The basic medium	Ph. mg/l	BA mg/l	21P mg/l	AIB mg/l
1.	V_o	MS(MB)		-	-	-
2.	V_1	MS	100	-	-	-
3.	V_2	MS	200	-	-	-
4.	V_3	MS	100	1.0	-	0,5
5.	V_4	MS	200	-	1,0	0,5
6.	V_5	MS	100	2,0	-	0,5
7.	V_6	MS	200	-	2,0	0,5

*MS = the medium after Murashige-Skoog; MB = the basic medium; Ph. = phloroglucinol; BA = benzyl adenine; 2iP = 2 - izopentyladenine; AIB = indolylbutiric acid.

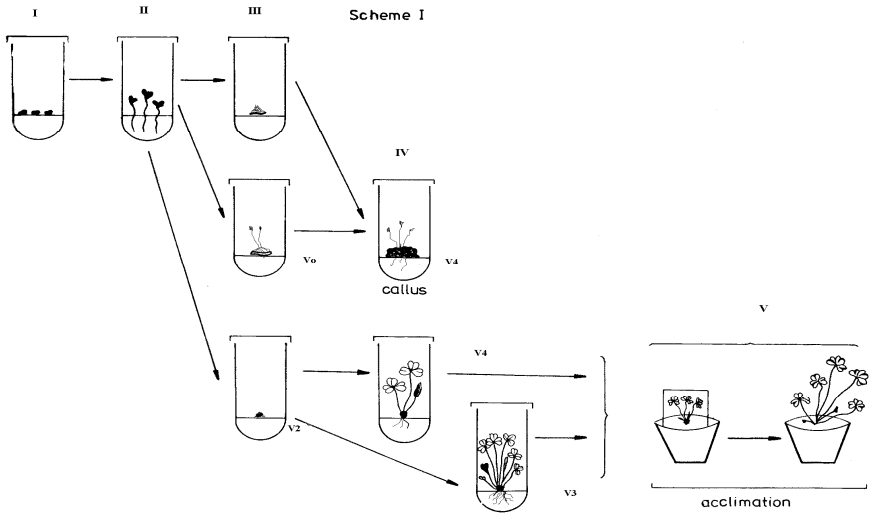


Fig.1. The stages of formation of the plantlets of clover in vitro

The plant material was formed by *apex* of clover detached from the neopantlets obtained by germination in vitro. Figure 1 show the stages of the *in vitro* organogenesis (I – V), depending on the hormonal balance, with aspects of acclimatization of the neoplantules

formed *in vitro*. We have replaced an equal number of explants/bottle for an efficient calculation of the average, the inoculums being kept in the conditions of the room of growth.

RESULTS AND DISCUSSION

The observations have been done after three months of culture in vitro. We pursued the percentage of regeneration in vitro, the number of neoplantlets and their length, the value of the radicular system formed, and a novelty element which appeared for the first time at the clover experiments initiated by us was the forming of the nodes. The results of our observations have been reproduced in Table 2. The percentage of regenerated explants was already analyzed after two months, and the other parameters after three months.

Table 2

The average values of the analyzed parameters at the culture of the clover apex in medium with phloroglucinol (after three months).

Var.	% of regeneration	Neoplantules No./Length (cm)		The radicular Systeme No.root/Lenght(cm)		Observations (the formation of nodosities)	Bonus
V_o	36	2	**8,0**	2	0,7		xx
V_1	55	3	5,0	3	0,9	2 nod./exp. of about 1mm	xxx
V_2	58	6	3,2	-	callus	1-2 nod/exp. of about 1mm	xxxx
V_3	**100**	**17**	**5,2**	8	10,0	3 nod./exp. of about 3mm	xxxxx
V_4	75	10	3,1	2	little callus	-	xxxx
V_5	**98**	12	4,9	5	9,5	5 nod./exp. of about 5mm	xxxxx
V_6	70	8	3,0	-	callus	-	xxx

Observing Table 2 and Figure 1 we can see that the maximum percentage of regeneration (100%) was recorded in the V_3 medium (100mgs/l Ph. + 1mg/l BA + 0,5 mgs/l AIB) and 98% in V_5 (with double dose of BA), in comparison to the control version (V_o) in which the percentage reached only to 36%. At the explants of clover cultivates into an medium where we added only phloroglucinol (V_1 and V_2), the in vitro regeneration reaches values of 55%, and 58%. Regeneration of clover plantlets are also completely organized in medium with 100mgs/l Ph. (Photo A). Figure 2 shows the average of the number of plantlets and their length at all experimented versions, distinguishing versions V_3 and V_5, on which we can obtain the best radicular system, presented graphically in figure 3.

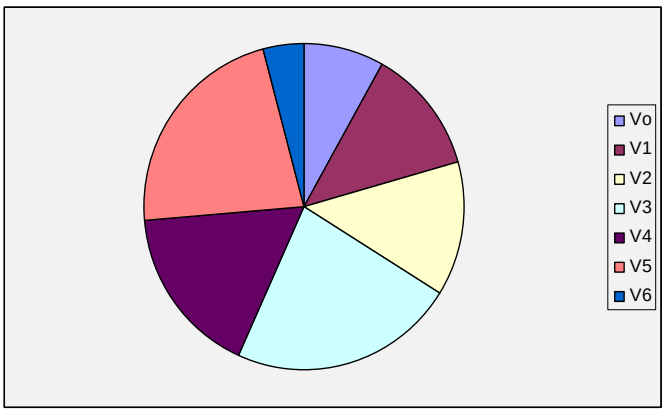


Fig.2 The regeneration percentage of the clover apex in medium with phloroglucinol

The clover explants cultivated in medium with double dose of phloroglucinol have formed a little number of neoplantlets and they did not initiate roots, but only a manson of callus around the plantlets, fact that determined them to stop rooting (see Table 2). The effect of the 2izopentiladenine in association with the phloroglucinol is favorable to clover regeneration and to its multiplication, but inferior to the benzyl- adenine.

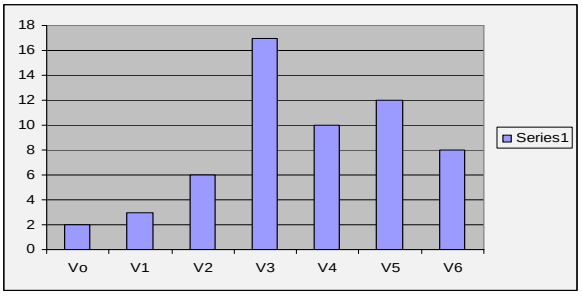


Fig.3 The average of the number of Trifolium pratense L. neoplantlets obtained in medium with phloroglucinol

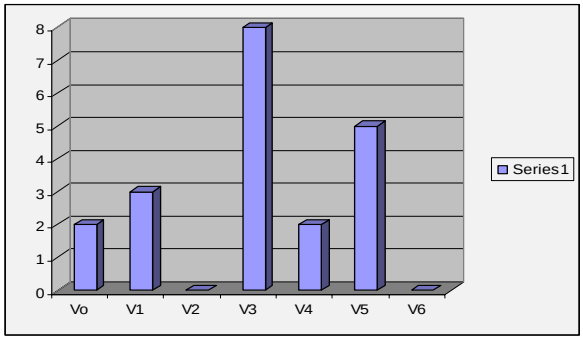


Figure 3. The number of roots from the clover apex formed in medium with phloroglucinol

Both on the radicular system and on the callus undesired nodes have developed after about four months, 2 or 3 nodes/explant with the diameter of 1-5 mms **(**Figure C**).** Their formation into an aseptically medium is an element of novelty in clover culture in vitro, this is why we consider that the composition of the medium and the time of year when we initiated the culture (May – April) to be responsible for the appearance of the nodes. They were formed, in a large part, into a medium with 100mgs/l phloroglucinol. We propose to follow closely this phenomenon particularly valuable in future studies.

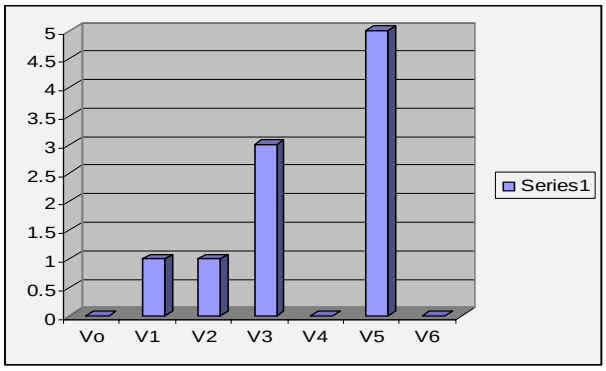
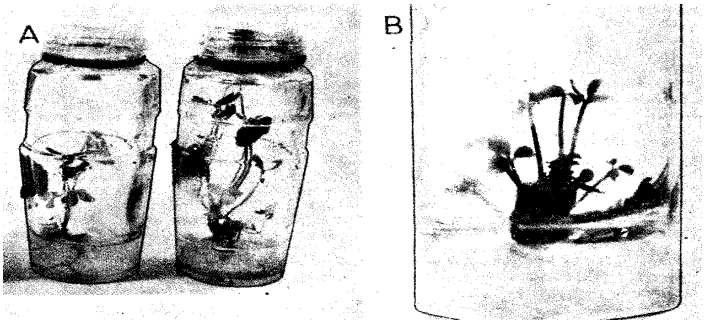


Figure 4. The formation of the nodes on the roots of clover neoplantlets cultivated in medium with phloroglucinol



A B.

A. The clover neoplantlets completely organized (into an medium with moderate dose of phlorogucinol and cytokinins);
B. Clover neoplantlets surrounded by a callus manson (into an medium with high dose of phloroglucinol)

C. Clover callus generated from the apex into an V_2 medium with formed nodes

CONCLUSION

1. The 100mg/l Ph. concentration combined with BA or 2iP (1 mg/l) and 0,5 mg/l AIB leads to maximum regeneration (100-98%) of the clover inoculums cultivated in vitro.
2. The regenerated plants on the versions with 100mg/l Ph. (V_3 and V_5) are completely organized from the point of view of the length of the neoplantlets formed and of the value of the radicular system.
3. The radicular system formed in vitro into an medium with 100mg/l Ph., achieves both in number, and in roots length the highest in values on the V_3 and V_5.
4. The double concentration of phloroglucinol (200mg/l) in the combinations specified in Table 1, (V_4, V_5), stimulate the regeneration of plants that are completely conformed to callus formation around them (photo B), what determines the inhibition of their roots.
5. The novelty element, the formation of nodes (about 2-5/explant), has favorably influenced the acclimatization.

REFERENCES

1. *V.M Kulcarni, , T.R.Ganapathi, P. Suprasanna and V.A. Bapat*.,2007, „In vitro mutagenesis in banana(*Musa spp*.) using gamma irradiation", in: Protocols for Micropropagation of Woody Trees and Fruits, Editat de S. Mohan Jain and H. Haggman, Springer, pp. 543-559.
2. *T. Murashige., Skoog, A*.,1962, Revised medium for rapid growth and bioassays with tabbacco tissue cultures, Physiol. Plant, 15, pp. 85-90.
3. *Savatti, M., Zăpârţan, M., Ienciu, A., Vicaş, G., Marele, D., Popovici, M., Popa, A., 2006*, Obtaining the genetical variability through mutagenoisis in vitro on red clover (Trifolium pratense L.) în: 41 croatian and 1 International Symposium on Agriculture, 13 – 17 Februaty, 2006, Opatija – Croatia, p. 229.
4. *Vicas Gabriela, 2008,* Rolul unor agenti chimici alchilanti in morfogeneza *in vitro* a unor specii.Implicatiile lor in ameliorare si ecologie, Agricultura nr.3-4, Cluj Napoca
5. *Vicas Gabriela, 2008,* Research on the effect of treatment with mutagen chemical agents over the formation process of red clover neoplants, Bul.USAMV, Cluj-Napoca, 65, pag. 330
6. *Zăpârţan M., Savatti, M, Ienciu, A., Buzaşiu, O., Vicaş, G. 2006.* Mutagenic effect of some chemical reagents on wite cloner (Trifolium repens L.) callus obtained in vitro. în: 41 Croatian and 1 International Symposium on Agriculture, 13 – 17 February Opatija – Croatia, p. 221.
7. *M., Zăpârţan., Deliu C.,* (1995), Efectul floroglucinolului în regenerarea şi multiplicarea in vitro a unor specii, Analele Univ. Oradea, Biologie, Tom II, pp. 36-42.
8. *M., Zăpârţan., Butiuc-Keul A., Deliu C., Deliu-Munteanu C.,* 1999-2000, Regenerative capacity of *Lilium longiflorum* Thumb. species cultivated in vitro, Contribuţii Botanice I, Grădina Botanică Alexandru Borza, Cluj-Napoca, pp. 131-137.
9. *M., Zăpârţan*, 2001, Conservarea florei spontane prin înmulţire in vitro, Ed. ALC MEDIA GROUP, Cluj – Napoca, pp.119 - 122
10. *M., Ziv., and Halevy, A. H.,* 1983, Control of Oxidative Browing and in vitro Propagation of *Strelizia reginae*, in: Hort Science, 18(4), pp. 434 – 437.