THE IN VITRO TUBERISATION AT THE POTATO DESIRÉE VARIETY IN MEDIUMS WITH PHLOROGLUCINOL

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Abstract. *The phloroglucinol, supplied in medium of culture, proved itself very efficient at some species which have a difficult reaction at the in vitro culture (strelizia, musa, magnolia, some species from the Liliaceae family, etc.).In the case of the specie studied by us,* ***Solanum tuberosum L****, the phloroglucinol added in the Murashige-Skoog(MS) medium, in dose of 100 and 200 mg/l, has stimulated the percentage of regeneration and formation of tubers at the potato* ***Desirée*** *variety. The explants consisting of the* ***apex*** *have been cultivated on the mediums specified in table 1, the results proving to be dependent of the medium's composition. The formation of the greatest number of tubers in vitro has been obtained on the variants with phloroglucinol, in the presence of the zeatine. On these mediums a small differentiation of the minitubers took already place after 30 days of culture in vitro. The neo formed minitubers in vitro reach until 4-5 mm in diameter only after another two months of culture in vitro (photo 6). We have followed the rate of the neo formation of potato tubers from the* ***apex*** *detached from the sprouts obtained by forcing the potato tubers. The percentage of tuberisation is of 50% after two months and of over 80% after three months on mediums with 100mg/l phloroglucinol with a plus of zeatine and 2izopentiladenine(table 2).On the other variants the percentage reaches about 20%, and on witness (C_o),it reaches only 10-15%. The double concentration of phloroglucinol (200mg/l) in variants C_4, C_6, C_8, also stimulates the regeneration of plants completely conformed to the corresponding root system. The time of tuberisation for those variants is of over three months. The place of formation of the minitubers is along the strains (especially in the node zone), but also at the basis of the plants.*

Key words: *phloroglucinol, apex, minitubers, tuberisation, neoformation, regeneration, organogenesis*.

INTRODUCTION

It is known a worth being noted the fact that the further additions in the aseptic mediums, for example the different doses of sucrose (7), the additional treatments through irradiation of cultures (8), different photoperiod (4) or bioactive magnetophluidic nanocomposites (5), etc., have favourable effects, especially over varieties which have a negative reaction at the classical medium experimented for their regeneration *in vitro*.

The phloroglucinol is a growth regulator from the 1,3,5 – trihydroxybenzen [$C_8H_3(OH)_3$ $2H_20$] group, which can be supplemented in the culture mediums for growth and development stimulation of some species of plants *in vitro* (11), with results depending of the substance concentration, of the nature of the specie and of the type of explant. Used on mediums of culture, it proved its efficiency in the regeneration *in vitro* of some species of plants which have no reaction in the classical aseptic medium. One of those species is *Strelizia reginae*, to which, the apex cultivation on mediums with phloroglucinol had remarkable results in terms of the regeneration and the multiplication in vitro (14). The same source states that at the Musa spp., which is reluctant at the *in vitro* multiplication, adding phloroglucinol in the medium of culture has proved efficient in concentration of 100mg/l(13).The 100 mg/l concentration has stimulated the *in vitro* regeneration and the bulbification at some species of Lilium(12), with superior results in combination with cytokinine (BA – in this case). If phloroglucinol is alone in the medium, the capacity of regeneration of the tissues is inferior in comparison with o small dose of cytokinine and auxine (10).

MATERIAL AND METHOD

The present study has proposed the following of the phloroglucinol effect over the tuberisation of potato Desirée variety cultivated in vitro, experimented either alone or in combination with cytokinine or auxine. The variety has been studied a lot under the aspect of the cytokinins implication in the differentiation of the *in vitro* minitubers (6). We have followed the capacity of formation of minitubers from the **apexes** detached from the *Solanum tuberosum L* specie *Desirée* variety, cultivated on mediums supplied with phloroglucinol. The basic medium used is the one after Murashige – Skoog- 1972(9), with the following composition: macro., microelements and FeEDTA – MS; mesoinositol – 100mg/l; thiamine HCl, pyridoxine HCl and nicotinic acid 1 mg/l; sucrose – 30 g/l; agar – 7 g/l; pH = 6,1 at this medium considered basic medium, we have added phloroglucinol in two concentrations: 100 and 200mg/l, either alone or in combination with phytohormones (BA, 2iP, Z, ANA), in the same dose (0,5 mg/l). Witness variant (C_o) was only the basic medium (MB) plus 5 g/l charcoal.

Table 1.

The composition of mediums supplied with phloroglucinol

Var.	MB	Ph. mg/l	BAmg/l	2iP mg/l	Z mg/l	ANA mg/l	charcoal
C_o	MSC	-	-	-	-	-	5 g/l
C_1	MS	100	-	-	-	-	-
C_2		200	-	-	-	-	-
C_3		100	0,5	-	-	0,5	-
C_4		200	0,5	-	-	0,5	-
C_5		100	-	0,5	-	0,5	-
C_6		200	-	0,5	-	0,5	-
C_7		100	-	-	0,5	0,5	-
C_8		200	-	-	0,5	0,5	-

(MB= basic medium; MS= after Murashige-Skoog medium; MSC= MB + 5g/l active charcoal; Ph= phloroglucinol; BA= benzyladenine; 2iP= izopentyladenine; Z = Zeatine; ANA= naphtyla acetic acid)

The *Solanum tuberosum L* specie was studied for a long time by our group from the point of view of the organogenesis (3) and of the one of *in vitro* tuberisation (1), with remarkable results, proving itself to be a specie with a high capacity of *in vitro* regeneration and multiplication, dependent on the variety, on the hormonal balance of the medium, on the nature and dose of phytohormones used (2).

The present experiment has been initiated in a bad time of year regarding the development of the potato both *in vitro* and *in vivo* (late autumn). We have resorted to a small dose of cytokinine and auxine for pointing out the effect of the phloroglucinol. The plant material formed of the apex (vegetable peak) which was detached from the potato shoots obtained through forcing of the tubers in the greenhouse, after the disinfection (in $HgCl_2$ 0,1%, for 10 minutes) and the detachment of the apex was passed on the mediums from table 1 (C_o – C_8). The bottles with inoculs have been kept in the conditions from the growth room at the temperature of 25-26^{o}C and a photoperiod of 16 hours of light from 24 hours.

RESULTS AND DISCUSSIONS

The observations have been made after two months of *in vitro* culture, in which we have followed the percentage of *in vitro* regeneration, the number of regenerated neoplantules, the formed value of the radicular system and the *in vitro* differentiation of minitubers. The last aspect of the *in vitro* tuberisation has been followed after 2,5 and 3,5 months from the initiation of the culture. The average of the results is presented in Table 2. The bonification is relevant for the establishment of the effects that phloroglucinol has over the *in vitro* behaviour of the potato *Desirée* variety.

Table 2.

The evolution of potato apex (Desirée variety) on mediums with addition of phloroglucinol

Var.	% Regener.	Avr. no. of pl.	Avr. no. of roots	Nr. de Tub./apex	% tuberisation	Time of tub. months	Bonific.
C_o	50	8	3,2	1,1	10	-	xx
C_1	50	10	4,8	2,8	29,8	2,5	xxx
C_2	60	12	6,1	2,9	20,9	3,5	xxx
C_3	70	20	10,0	7,5	70,5	2,5	xxx
C_4	**100**	25	18,8	4,9	41,0	3,5	xxx
C_5	70	35	12,0	**9.0**	**90,0**	2,5	**xxxxx**
C_6	**100**	**40**	**19,0**	7,8	71,0	3,0	xxxx
C_7	**100**	35	18,5	**9.9**	**99,1**	2,5	**xxxxx**
C_8	**100**	**40**	**30,5**	7,5	70,0	3,5	xxxx

The percentage of regeneration is generally good on all mediums (50-70%), but on the variants with Z and 2iP combined with phloroglucinol, the percentage riches 100% (Fig.1).

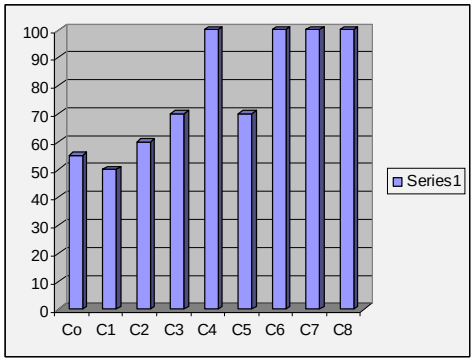

Fig. 1. The percentage of the in vitro regeneration of the potato apex, *Desirée* variety (after two months)

The average of the number of plants regenerated in vitro, has reached about 35-40 of plants/apex at the mediums with phloroglucinol, Z and 2iP (C_5 – C_8), between 20-25 plantules/apex on the variants with phloroglucinol (C_3 and C_4), about 10 – 12 % on the mediums with Ph only (C_2 – C_3) and 8 plantules/apex per witness (C_o) (see fig.2).

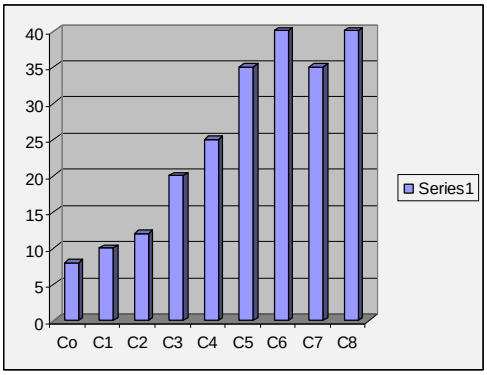

Fig.2. The average of the number of potato neoplantules regenerated *in vitro* (after 2 months)

The average of the number of roots is between 18 – 30 of roots/apex, at the variants with phloroglucinol +Z +2iP and 10-18 roots/apex on the other variants, their length reaching about 4-8 cm. On mediums with Ph only the average of the number of roots is greater, 4,8 – 6,1 roots/apex, and on the witness of about 3(Fig.3).

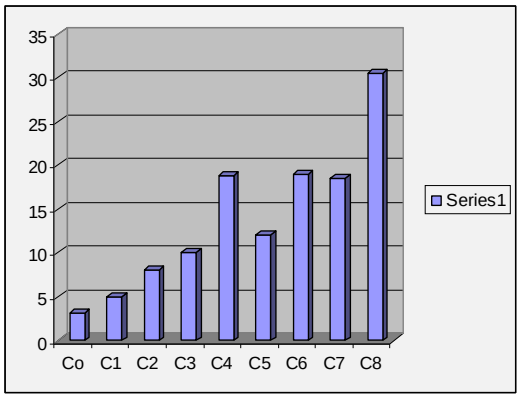

Fig. 3 The average of the number of roots obtained on mediums with phloroglucinol (after 2 months)

The number of tubers per explant has been followed after 2,5 months of *in vitro* culture. On the variants with a small dose of phloroglucinol, the number of tubers is superior to those differentiated on mediums with greater dose (200 mg/l Ph), on the latter, the tuberisation shows up in a good percentage only after a month (about 3,5 months). The tuberisation process has been induced on all variants, but different as a number (fig.4), size and form of minitubers (photo 6).

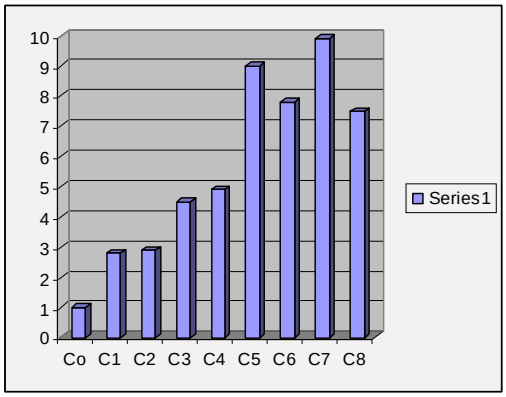

Fig. 4 The average of the number of tubers/ explant at the *Desirée* variety (after 2,5 and 3,5 months)

The percentage and time of tuberisation is presented in fig. 5 and table 2, from which we can see that on the variants with a high dose of phloroglucinol, the percentage of tuberisation is of about 70% and it takes place after a long period of time (3,5 months), in comparison with the ones on the variants with a small dose, to which the percentage of tuberisation reaches up to 90-99% , the time of tuberisation being shorter (2,5 months).

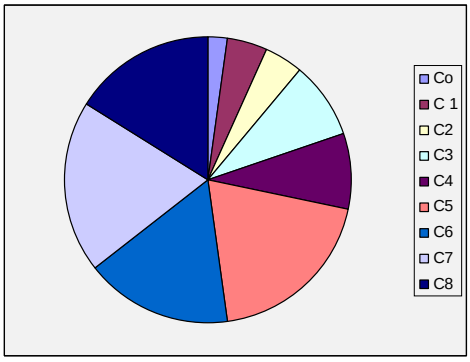

Fig. 5. The percentage of minitubers on variant (after 2, 5 and 3, 5 months)

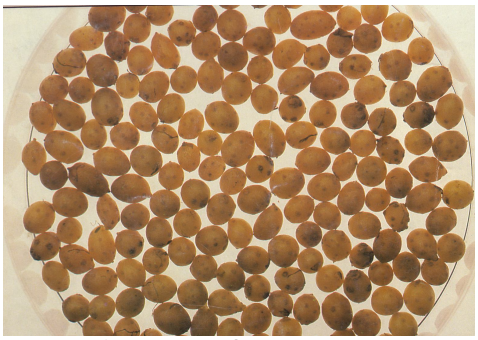

Fig.6 The form and the aspect of potato *Desirée* minitubers obtained *in vitro* on mediums with phloroglucinol and phytohormones (after about 3,5 months)

CONCLUSIONS.

1. The phloroglucinol in presence of cytokinines (C_4,C_6,C_7 and C_8) determines a 100% percent of regeneration.

2. The great concentration of phloroglucinol (200 mg/l) in presence of the Zeatine and of the 2iP, stimulates the organogenesis, developing about 40 well organised neoplantules/apex.

3. The greatest number of roots is obtained on the mediums with a high concentration of phloroglucinol + zeatine, about 18-30 roots/apex (C_6), but the rooting process is also stimulated on the other variants.

4. We recommend adding phloroglucinol in the Murashige – Skoog medium, at the *in vitro* potato cultures, in moderate dose of 100 mg/l for stimulating the *in vitro* formation of tubers.

5. The phloroglucinol, in medium of culture combined with a small concentration of cytokinine or auxine (about 0,5 mg/l) has proved to have a beneficial effect.

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