

ECONOMICAL METHODS OF *IN VITRO* TUBERIZATION AT *SOLANUM TUBEROSUM* L VARIETY

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Abstract

The present paper aims in vitro culture of two potato varieties Desirée and Ostara on the medium in which the growth hormones were replaced with natural extracts, using corn germ extract and yeast in three concentrations 1, 3 and 5mg/l. We have followed the percentage of regeneration of the potato apex, the percentage of rooting of the neoplantlets in vitro and the aspects of rooting, compared to the control medium MS (V₀), without hormones and hormone-free medium MS +BA- 1 mg/l + AIB – 0.5 mg/l (V₁). At Desirée variety on media with extracts of corn germ, the highest concentration of 5 mg/l (Ep₃ variant) has generated a percentage of regeneration of about as big as on the variant with hormones, of 90 %. On the other variants the percentages are smaller. In the same way, the yeast extract has given a smaller percentage of regeneration, but relatively good also on the high concentration of 5 mg/l (Ed₃) of 45%. Ostara variety presents an inferior reaction even to high concentration of corn extract, of 5 g/l, the regeneration reaching only 50%, and on the yeast extract of 35%. The rooting of Desirée variety has proved to be good on medium with 5mg/l corn extract, up to 30%,(about 6-7 tubers/apex), compared with the other concentrations and with the other variety. The replacement of hormones from the medium with natural extracts has beneficial and economical effects, the results depending on the concentration of the extract and on the nature of the soil.

Key words: apex, *in vitro Solanum tuberosum* L, Desirée, Ostara, corn germ extract (Ep.), yeast extract (Ed), regeneration, rooting.

INTRODUCTION

It is known that investigations are ongoing concerning the knowledge of explants responsiveness or of inoculums at different compositions of the culture medium, sometimes new technical or economic solutions are discovered, some of them specific for some types of cultures (Keul A., Deliu C, 2000). Complex substances and natural plant extracts are generally used in low concentration of about 1 g/l. Their effect was also tested in high doses (of 3 – g/l) which has proved to have a stimulating effect in the *in vitro* organogenesis, due to the intake endogenous hormones, natural vitamins, amino acids, and to some active compounds easily assimilated and to some chemical elements, linked in complex easily accessible plant cells (Cachiță, 1987).

Lengthy experiments on *Solanum tuberosum* L varieties have proved that further additives in the culture medium are beneficial for the *in vitro* evolution. Agud, E et al., 2009 and 2010, have showed the same thing at a larger number of potato varieties grown *in vitro* and in several experimental series. A balanced balance of phytohormones in the potato's culture medium

(Butiuc-Keul, A., et al., 1997 – 1998) can ensure a superior regeneration and minituberization. In vitro behavior of potato varieties is influenced by a lot of other factors, for which the variety's biological value, the line from which the explant was detached, the time of year when the culture is initiated, the contribution of endogenous hormones (Butiuc – Keul A., et al., 1996.), the temperature, the season, the photo-period (Agud, E., et al., 2008), the minerals from the medium and the hormonal balance added to the medium, all those factors play a decisive role in obtaining the desired results at potato varieties (Baciu, A. 2008). Micro propagation of species of economic interest, by using automated devices, robots and economical culture media (Cachiță-Cosma, D., et al., 2007), is an area of interest.

MATERIALS AND METHODS

The success of the economical in vitro culture at the potato variety is also due to the replacement of synthetic hormones from the medium with natural extracts. Thus, in the present paper we resorted to the use of corn germ extract and yeast in the culture medium, on which was inoculated the apex from potato *Desirée* and *Ostara* varieties. The extract of corn germ was successfully used in the in vitro regeneration and multiplication of some plant species: *Leontopodium*, *Dianthud*, *Chrysanthemum*, *Daphene*, *Drosera*, *Fritillaria* (Zăpârțan, M., 1995; Zăpârțan, M., Deliu, C. 1995; Zăpârțan, M. 1996, a, b and c; Butiuc, A. and Zăpârțan, M. 1996). The extract of yeast was especially used for callus induction from flower organs (Yamagishi, M., 1995).

We were seeking to obtain in vitro an appropriate biological material, organogenetic and evolutionary, ensuring in vitro tuberization at the mentioned varieties. To this end, apex was detached from shoots from the varieties cultivated in vitro and they were inoculated on the variants of medium presented in table 1. Culture media were adjusted at a Ph = 5,7 before autoclaving at 1 atmosphere and 120°C, for 20 minutes.

Table 1

Medium variants with natural extracts for the culture of potato varieties			
Variant	Medium composition	Corn extract (Ep.)	Yeast extract (Ed.)
V ₀	MS	-	-
V ₁	MS + BA-1mg/l + AIB – 0.5 mg/l	-	-
Ep ₁	MS	1.0 mg/l	-
Ep ₂	MS	3.0 mg/l	-
Ep ₃	MS	5.0 mg/l	-
Ed ₁	MS	-	1.0 mg/l
Ed ₂	MS	-	3.0 mg/l
Ed ₃	MS	-	5.0 mg/l

(MS = basic medium after Murashige – Skoog- 1962; Ep = corn germ extract; Ed = yeast extract; BA = benzylaminopurine; AIB = butyric acid indolil)

RESULTS AND DISCUSSION

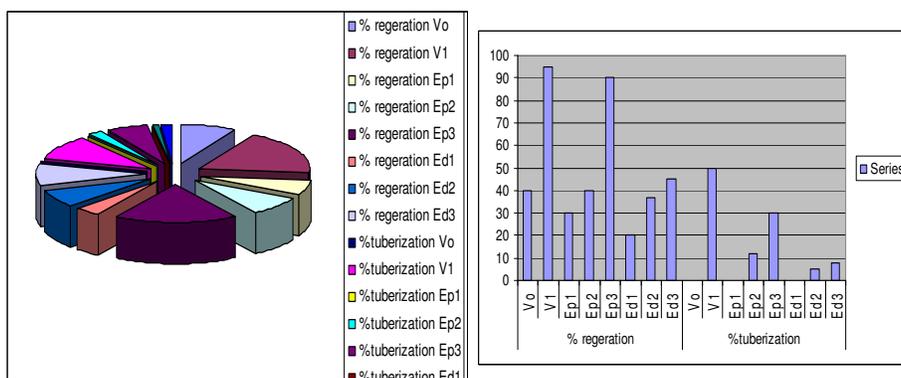
Cultures' assessment was made after 5 weeks after inoculation, aiming at the percentage of regenerated, rooted and tubered explants, and also the number of minitubers obtained in vitro on an apex. The results of the observations at the two potato varieties are included in table 2. Following the table we can see from the beginning the superiority of the variant with hormones (V_1), and also for *Desirée* variety, but at the same time we see the beneficial effect of the corn germ extract in the medium, especially in a high concentration of 5mg/l. Parameter values on these concentrations are close to the ones obtained on variant V_1 with growth hormones (see table 2).

The behavior of *Desirée* variety on media with natural extracts is superior to Ostara variety. Fig. 1 a and b present the evolution of *Desirée* variety on all medium variants, of control (V_0 and V_1) and with corn extracts (Ep_1 , Ep_2 and Ep_3) and with yeast extract (Ed_1 , Ed_2 and Ed_3).

Table 2

The evolution of potato apex on media with natural extracts (after 5 weeks)

Variant	% Regeneration		% Rooting		% Tuberation		No. of tubers	
	Desirée	Ostara	Desirée	Ostara	Desirée	Ostara	Desirée	Ostara
V_0	40	23	38	23	-	-	-	-
V_1	95	59	80	50	50	10	6	3
Ep_1	30	18	20	10	-	-	1	-
Ep_2	40	20	28	15	12	-	2	-
Ep_3	90	55	85	50	30	5	8	2
Ed_1	30	20	30	20	-	-	-	-
Ed_2	37	27	35	25	5	-	2	-
Ed_3	45	35	42	35	8	3	3	1



a.

b.

Fig. 1 Regeneration and tuberization percentage at *Desirée* variety on media with natural extracts (Ep = corn extract, Ed = pumpkin extract)

Following fig 1 b we find that the regeneration percentage on medium with hormones (V_1), reaches the same values (about 95 – 90%) as on the medium with the maximum concentration of corn extract (Ep_3). The evolution of this parameter on the other variants is inferior and the values are between 20 – 40%. The percentage of tuberization is also the highest on variants V_1 and Ep_3 , between 30 – 40%, and on the other variants is weak or nonexistent.

The reaction of *Ostara* variety on media with natural extracts is inferior to *Desirée* variety. Fig. 2 a and b show the evolution of *Ostara* variety on all variants of medium, of control, with added hormones (V_0 and V_1), with corn extracts (Ep_1 , Ep_2 and Ep_3) and with yeast extracts (Ed_1 , Ed_2 and Ed_3). From fig. 2 a we can see that at this variety the percentage of regeneration on medium with hormones (V_1), reaches values of 55%, and on the medium with maximum concentration of corn extract (Ep_3), a percentage of 50%. The evolution of this parameter at *Ostara* variety on the other variants is inferior, between 18 – 27%. Tuberization percentage is reported only on variants V_1 , 10% and on Ep_3 about 5%, and on the other variants the percentage is low or they did not differentiate at all any tubers in vitro.

The correlation between the two parameters (regeneration and tuberization) shows that coefficient values have revealed the fact that there was achieved a significant positive correlation on medium with $Ep - 5 \text{ mg/l}$ between regenerative capacity and tuberization in vitro both at variety *Desirée* and *Ostara*, the values of the last one being inferior.

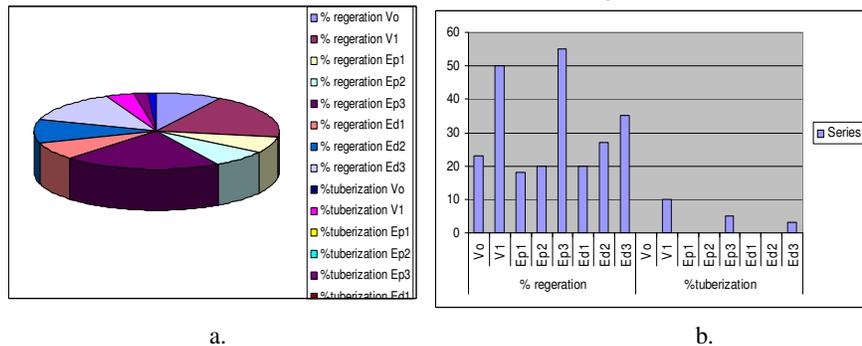


Fig. 2 The regeneration and tuberization percentage at *Ostara* variety on media with natural extracts

The results at potato varieties reveal that the natural extracts, due to their rich content in vitamins (B and E), nitrogenous substances, carbohydrates, lipids, phosphorus, iron, etc., may positively influence the processes of regeneration and organogenesis at plants cultivated in vitro.

CONCLUSIONS

1. Natural extracts added to the media of culture may supplement the actions of the usual components of the culture medium, or may completely replace some of those components that are more expensive, as for example phytohormones, reducing the cost price of the micro propagation of some plants.

2. The results obtained at potato variety are similar to the ones obtained on medium supplemented with hormones. *Desirée* variety has a reaction superior to *Ostara* variety, and the effect of the corn germ extract is superior to the one caused by the yeast extract.

3. The natural extract of corn germ ensures *Desirée* potato variety with the obtaining of a greater number of neoplantlets (90 neoplantlets/apex) with good rooting and a good percentage of tuberization (30%), superior on the medium with high concentration of extract (5mg/l).

4. Natural extracts at *Ostara* variety have an inferior effect in regeneration, rooting and tuberization (somewhat better on high concentration), which shows the effect dependent on the variety and on the concentration of the extract.

5. Although some results are slightly inferior to the ones obtained on media with phytohormones, we can recommend replacing the more expensive constituents from the culture medium, with natural extracts in order to reduce the cost price, but taking into consideration the specie, the variety and the concentration of the extract.

We recommend the use of natural extracts in the culture medium, for the stimulation of in vitro regeneration and tuberization. The results depend on the variety, on the nature of the extract and on its concentration. *Desirée* potato variety responds best to the maximum concentration of natural extracts (5mg/l) introduced in the culture medium, results similar to the ones on media with hormones. We note, in this experiment, the absence of some secondary undesired phenomena, as for example necrosis and vitrification, common for the in vitro culture of potato.

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