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EFFECT OF ANTIVIRAL COMPOUNDS ON THE REGENERATION OF POTATO MERISTEMS

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ABSTRACT

The aim of this research was to determine the phytotoxic effect of *chemotherapeutic products* over meristematic explants from two potato Romanian varieties: Marvis and Castrum. Potato meristems were cultured on nutritive medium containing four virocid chemicals: ribavirin, 5-bromouracil, 2-thiouracil and acyclovir in three concentrations (0, 15, 30 mg/l). Meristems development varied for tested antiviral agents and was decreased with increasing in concentration of antiviral products (from 0 to 30 mg/l). Antiviral products with high concentration (30 mg/l) had a negative effect on regeneration. By using 5-bromouracil the highest regeneration rate was observed (for 15 mg/l and 30 mg/l), compared to the other antiviral substances, with the same concentrations. Another chemotherapeutic 2-thiouracil exhibited phytotoxicity and reduced meristems regeneration. The lowest percentage of regeneration was registered by using of 15 mg/l 2-thiouracil compared to the other antivirals, at the same concentration. Percent of meristems regeneration was very low for ribavirin and 2-thiouracil at 30 mg/l concentrations (40%). For Castrum variety 5-bromouracil is distinguished by obtaining a very significant positive difference (19.17% regenerated meristems), but the virocid 2-thiouracil is at the opposite pole, inhibiting the regeneration of explants, with a very significant negative difference (-14.17% developed explants).

Keywords: potato, meristem culture, chemotherapy, antiviral agents.

INTRODUCTION

For seed potatoes production is essential using a healthy and high quality biological material, so can be obtain an increasing in potato production at an optimal level (Parrot, 2010).

The application of tissue culture and rapid propagation method for potato production continues to become more widely used in both developed and developing countries (Farhana Rumzum, 2013).

Meristem culture is a widely used method for virus eradication from horticultural plants (Faccioli, 2001, quoted by Vivek and Modgil, 2018). Mostly the apical meristems are often virus free but some viruses are known that invade the meristematic region of the growing tips (Vivek and Modgil, 2018). Faccioli (2001) presented that by using *in vitro* meristems with the primordial leaves pair, plantlets free of PLRV, PVY and PVM viruses in 100% was obtained and of PVX and PVS viruses in 66%. By inoculating small meristems (0.1 mm) with or without the primordial leaf pair, a high rate of elimination of PVX and PVS viruses was obtained, but only 20 plantlets regenerated from 196 meristems (Kassanis and Varma, 1967). Potato viruses A (PVA) and Y (PVY) were eliminated in 85-90% using meristems smaller than 0.3 mm (Morel et al., 1968), while other viruses (PVX, PVS) did not could be eradicated.

Meristem culture along or in combination with other methods have become a powerful and successful tool for virus elimination from infected plants (Paet and Zamora, 1990).

One of the most viable methods for obtaining virus-free stocks from propagative materials that comes from infected plants is viral eradication by using tissue culture techniques, aided or not by chemotherapy (Awan et al., 2007; Mellor and Stace-Smith,

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1970). Meristem culture and chemotherapy allow quick propagation of plant materials, producing healthy plants in a short period of time. Klein and Livingston (1983) presented that, Potato Virus X (PVX) and Potato Virus Y (PVY) were eliminated by meristem tip culture and chemotherapy using ribavirin, but the time required for regeneration of the tips was longer than the untreated controls.

Chemotherapy directed to plant viral diseases has evolved significantly. The most frequently studied compounds are antimetabolites, substances capable of blocking the virus nucleic acid synthesis (Vicente and De Fazio, 1987). To eliminate potato viruses, chemotherapy, especially with ribavirin, was widely applied alone or combined with other methods such as meristem culture, cryotherapy, electrotherapy and cryotherapy (Hu et al., 2015; Singh, 2015; Chahardehi et al., 2016; Kushnarenko et al., 2017). Chemotherapy applied into culture medium can affect tissue development and meristems growth, inhibiting shots regeneration from meristems (Loebenstein et al., 2001).

This research analyzes chemotherapy role over meristem culture regeneration and identification the best antiviral agent with less phytotoxicity effect.

MATERIAL AND METHODS

The study was performed at Laboratory of Vegetal Tissue Culture of the National Institute of Research and Development for Potato and Sugar Beet Brasov and two important Romanian cultivars, created at our Institute are analyzed. The influence of antiviral compounds on meristems regeneration is analyzed.

For meristem culture, active growth shoots were used. They were sterilized, followed by meristematic dissection. This procedure is very delicate and all operations were performed in the sterilized chamber, in the niche with laminar flow of sterile air, previously sterilized. For the virus's eradication, the meristematic explant was constituted from meristematic dome, together with one or two of the primordial leaf pairs.

The success of the virus depends on the size of the meristem, but the smaller they are, there is a risk of necrosis of these. Because the size of the excised meristems, the cultivars studied and the type of virus are the main factors that often influence the success in eliminating the plant virus, it was tried to add different antiviral substances in the culture medium, in order to obtain a greater success in achieving biological material free of viruses. Because the meristematic explants were very small (0.2-0.3 mm) and under the influence of chemotherapeutics agents, there may be a higher risk of necrosis, for the beginning, standard medium Murashige-Skoog (MS) (1962) was used for the first meristems inoculation. Meristematic explants were inoculated *in vitro* on aseptic nutritive medium immediately after sizing. In a single-factor experience, on six repetitions, after the first inoculation, one month later, the percentage of regenerated meristems was determined, depending on the varieties studied: Marvis and Castrum. For a greater success of virus elimination was established four antiviral agents with different concentration. These were added to the standard culture medium with 0.5 mg/l gibberellic acid (AG_3), 20 g/l sucrose, 9 g/l agar (pH adjusted to 5.8 before autoclaving with 1N NaOH or 1N HCl). Three subcultures were done at a 30 days interval, respecting the same types of culture medium. After this period was evaluated the explants regeneration rate. Statistical interpretation of the obtained results was made using analysis of variance method (Săulescu N.A. and Săulescu N.N., 1967). Trifactorial experience, 2x4x3, with 24 variants, divided into 5 repetitions included the following factors: the experimental factor A - the variety with 2 graduations: a_1 - Marvis and a_2 - Castrum; the experimental factor B - the antiviral product, with four graduations: b_1 - ribavirin; b_2 - 5-bromouracil; b_3 - 2-thiouracil; b_4 - acyclovir; the experimental factor C - concentrations of antiviral products, with three graduations: c_1 - 0 mg/l (standard medium Murashige - Skoog consider control); c_2 - 15 mg/l; c_3 - 30 mg/l.

RESULTS AND DISCUSSION

Analysis of variance for the meristems regeneration (Table 1) shows a significant positive difference for the Marvis variety (2.5%) compared to the values mean (considered control).

The influence of antiviral products on the meristem regeneration (%) of the varieties studied shows the superiority of the Castrum variety (74.17%), followed by the Marvis variety (71.67%) (Table 2).

Antiviral compounds, used in experience to observe their effect in the regeneration rate of meristems, have influenced the regeneration of the meristems. Thus, the difference of 13.75%, obtained when using 5-bromouracil was very significant positive (Table 3). The antiviral 2-thiouracil have a negative effect on the meristem regeneration rate, expressed by a very significant negative difference (-9.58%), compared to the mean values obtained (control).

Research on the influence of antiviral compounds concentrations on meristem development (Table 4) shows that by using concentrations of 15 and 30 mg/l (viral agent) wass registered a negatively influences on meristem regeneration rate, leading to a decrease from 100% to 47.5% (with very significant differences: -28.75% and -52.5%).

Table 1. Regenerative capacity of meristems after the first inoculation on the standard medium

Variety	Regeneration capacity (%)	Diff. (%)	Sign.
Marvis	90	2.5	*
Castrum	85	-2.5	o
Mean (Ct)	87.5	2.5	-

LSD: 5% = 2.20%; 1% = 3.45%; 0.1% = 5.87%

Table 2. Regeneration of meristems (%) from the varieties studied

Variety	Regeneration of meristems (%)	Diff. (%)	Sign.
Marvis	71.67	-1.25	ns
Castrum	74.17	1.25	ns
Mean (Ct)	72.92	-	-

LSD: 5% = 5.47%; 1% = 12.64%; 0.1% = 40.22%

Table 3. The influence of antiviral compounds on meristem regeneration (%)

The antiviral agent	Regeneration of meristems (%)	Diff. (%)	Sign.
Ribavirin	68.33	-4.58	o
5-Bromouracil	86.67	13.75	***
2-Thiouracil	63.33	-9.58	ooo
Acyclovir	73.33	0.42	ns
Mean (Ct)	72.92	-	-

LSD: 5% = 3.53%; 1% = 4.79%; 0.1% = 6.42%

Table 4. Influence of antiviral compounds concentration on meristem regeneration (%)

Concentration of antiviral agent (mg/l)	Regeneration of meristems (%)	Diff. (%)	Sign.
0 (Ct medium MS standard)	100	-	-
15	71.25	-28.75	ooo
30	47.5	-52.5	ooo

LSD: 5% = 15.00%; 1% = 20.22%; 0.1% = 26.83%

From the study of the combined influence of the varieties and antiviral compounds used (Table 5), it is evident for Marvis variety and also for Castrum variety, the positive influence of the 5-bromouracil antiviral agent, with the increasing of the meristem regeneration rate (%), and distinctly significant, respectively very significant differences (8.33% and 19.17%).

Statistical analysis of the differences obtained, compared with the calculated limit differences, in the case of establishing the variety's capacity over meristems regeneration, when was used the same antiviral agent, highlights the Castrum variety, which compared to the Marvis variety, is favored by the use of 5-bromouracil, obtaining a rate higher regeneration, with a difference of 13.33%, considered distinctly significant.

The concentration increasing of the antiviral agent, reflected in the regeneration rate of the meristems, for the varieties analyzed (Table 6), causes the decreasing of the regeneration process, therefore is obtaining distinctly significant differences or very significant negative differences for the Marvis variety (-30% and -55% respectively). For Castrum variety the regeneration rate is higher than for Marvis variety, but with a decreasing negative of values from 72.5 to 50.0% (with significant respectively very significant differences at concentrations of 15 and 30 mg/l).

Table 5. Combined influence of varieties and antiviral compounds on meristem regeneration (%)

Variety/Antiviral agent	Marvis variety (%)	Diff. (%)	Sign.	Castrum variety (%)	Diff. (%)	Sign.	a_2-a_1	Sign.
Ribavirin	66.67	-5.00	ns	70.00	-4.17	ns	3.33	ns
5-Bromouracil	80.00	8.33	**	93.33	19.17	***	13.33	**
2-Thiouracil	66.67	-5.00	ns	60.00	-14.17	ooo	-6.67	o
Acyclovir	73.33	1.67	ns	73.33	-0.83	ns	0.00	ns
Mean (Ct)	71.67	-	-	74.17	-	-	-	-
LSD: 5% = 5.28%; 1% = 7.41%; 0.1% = 10.46%				LSD: 5% = 6.53%; 1% = 11.58%; 0.1% = 27.59%				

The analysis of variance made to establish the combined influence of the antiviral agents' concentration on the regeneration of meristems, between the two cultivars studied, shows that the differences between them are insignificant, so the varieties have the same behavior, when the concentration of the antiviral agent increases (Table 6).

The combined influence of the antiviral agent and its concentration (Table 7) shows that using of the maximum concentration (30 mg/l) leads to a drastic decreasing in the rate of regeneration, from significant differences, to very significant negative (-40% and -60%, respectively for 5-bromouracil or ribavirin and 2-thiouracil). From the study of the differences obtained within the same concentrations for each antiviral agent, compared with ribavirin, there is a significant positive difference when is used the concentration of 15 mg/l, in the case of 5-bromouracil, compared to control medium (a difference of 35%).

The combined influence of the antiviral agents, concentrations and varieties, statistically analyzed in Table 8 is expressed by distinctly significant negative or insignificant differences. This indicates that increasing the concentration of the antiviral agent is not beneficial for meristems regeneration. It is observed that acyclovir (15 and 30 mg/l) has a less negative effect for the Marvis variety, with insignificant differences (-40%) compared to the control (medium MS). Also, 5-bromouracil (15 and 30 mg/l) has the same effect for Castrum variety, with insignificant differences compared to the control, standard medium of culture (0; -20%).

Table 6. The combined influence of the antiviral agents concentration and the variety on the meristem regeneration (%)

Variety/Antiviral agent concentration (mg/l)	Marvis variety (%)	Diff. (%)	Sign.	Castrum variety (%)	Diff. (%)	Sign.	a_2-a_1	Sign.
0 (Ct)	100	-	-	100	-		-	-
15	70	-30	oo	72.5	-27.5	o	2.5	ns
30	45	-55	ooo	50	-50	ooo	5	ns

LSD: 5% = 21.21%; 1% = 28.59%; 0.1% = 37.94% LSD: 5% = 17.94%; 1% = 24.96%; 0.1% = 36.60%

Table 7. Combined influence of varieties and concentration of antiviral compounds on meristem regeneration (%)

Variety/Con. antiviral agent (mg/l)	Ribavirin (%)	Diff. (%)	Sign.	5-Bromour. (%)	Diff. (%)	Sign.	2-Thioura. (%)	Diff. (%)	Sign.	Acyclovir (%)	Diff. (%)	Sign.	b_2-b_1	Sign.	b_3-b_1	Sign.	b_4-b_1	Sign.
0 (Ct)	100	-	-	100	-		100	-		100	-	-	0	-	-	-	-	-
15	65	-35	o	100	0	ns	50	-60	ns	70	-30	o	35	*	15	ns	5	ns
30	40	-60	ooo	60	-40	o	40	-60	ooo	50	-50	oo	20	ns	0	ns	10	ns

LSD: 5% = 29.99%; 1% = 40.43%; 0.1% = 53.66% LSD: 5% = 24.74%; 1% = 33.36%; 0.1% = 44.28%

Table 8. The combined influence of varieties, antiviral agents and their concentration on meristem regeneration (%)

Variety/Antiviral agent/Conc. (mg/l)	Conc. of antiviral ag. (mg/l)	Marvis variety (%)	Diff. (%)	Sign.	Castrum variety (%)	Diff. (%)	Sign.	$a_2b_1c_1$-$a_1b_1c_1$	Sign.
Ribavirin	0 (Ct)	100	-	-	100	-	-	-	-
	15	60	-40	ns	70	-30	ns	10	ns
	30	40	-60	oo	40	-60	oo	0	ns
5-Bromouracil	0 (Ct)	100	-	-	100	-	-	-	-
	15	100	0	ns	100	0	ns	0	ns
	30	40	-60	oo	80	-20	ns	40	ns
2-Thiouracil	0 (Ct)	100	-	-	100	-	-	-	-
	15	60	-40	ns	40	-60	oo	-20	ns
	30	40	-60	oo	40	-60	oo	0	ns
Acyclovir	0 (Ct)	100	-	-	100	-	-	-	-
	15	60	-40	ns	80	-20	ns	20	ns
	30	60	-40	ns	40	-60	oo	-20	ns

LSD: 5% = 42.41%; 1% = 57.18%; 0.1% = 75.89% LSD: 5% = 35.49%; 1% = 48.24%; 0.1% = 65.78%

CONCLUSIONS

Regarding the meristem's regeneration, analyzed varieties: Marvis and Castrum, behaved differently, depending on nutritive medium, antiviral product and their concentrations.

The influence of antiviral substances on the meristematic regeneration capacity was between 86.67% (5-bromouracil) and 63.33% (2-thiouracil).

The statistical analysis performed to establish the influence of antiviral substances concentration shows superiority for the standard medium MS, regarding the regenerations of meristems. Concentrations of 15 and 30 mg/l cause a very significant negative difference, compared to standard medium (control). So, increasing the concentration of the antiviral agent had a phytotoxicity for explants regeneration for both concentrations.

Regarding the meristematic regeneration rate, from the comparison of the results obtained between the varieties when using these 4 chemicals, the superiority is found for Castrum variety (93.33%), followed by Marvis (80.00%), by using 5-bromouracil.

The analysis of the influence of antivirals, depending on their concentrations,

shows that the meristems had a better regeneration by using 5-bromouracil, compared to ribavirin, for the concentration of 15 mg/l (100%).

In the future, research will continue on the influence of antiviral substances regarding the potato virus's elimination.

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