BENEFICIALTREATMENTS ON PVX AND PVY INFECTED POTATO (*SOLANUM TUBEROSUM* L.) PLANTS

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ABSTRACT

This study presents the efficiency of some combined techniques (chemo- and electrotherapy) in decreasing the infection level of PVY and PVX infected plants (cv. Roclas). The infected plantlets were exposed to 100 mA for 5, 10 and 20 minutes (electrotherapy), washed, divided into single node cuttings and multiplied in vitro. Chemotherapy was undertaken with ribavirin (RBV) and oseltamivir (OSMV). Solanum tuberosum L. plantlets regenerated were removed from the culture medium, acclimated in green house. The survivor plants were indexed (DAS ELISA, Bioreba, Switzerland). Distinguished virus elimination rates were obtained for all the material infected, using the most severe variants of electrotherapy (100mA/10minutes; 100mA/20 minutes). The highest values were registered in case of PVX infected material.

INTRODUCTION

Potato virus X (PVX), a *Potexvirus*, is present sometimes in commercial stocks of most varieties. Unfortunatly, it is responsible for some of the uncertainties encountered in field inspections. This virus is most dangerous when *Potato virus Y* is present because the synergy between these two pathogens causes severe symptoms in potatoes.

Potato virus Y (PVY), member of the *Potyviridae* is a dangerous pathogen for solanaceous crops. Beeing one of the the most economically disease problem in seed potatoes in many areas of the world, this virus has received an important attention. Potato virus Y is responsible for decreases in yield and quality, but the requirement for strict virus tolerance limits for certified seed is a hugge problem in seed potato production. Yearly, many lots are rejected as certified seed because their high levels of this virus, resulting in a significant reduction in crop value and in a shortage of certified seed, especially of certain cultivars that are highly susceptible to PVY infection [16].

Elimination of PVY and PVX from potato supply is essential for seed potato production. Also, in this study, the efficiency of some techniques (chemotherapy, electrotherapy) in eliminating PVY and PVX and producing virus-free plants (cultivar ‘Roclas’) was evaluated.

Untill now, many compounds were tested for their antiviral activity but few were effective [14]. The most used substance is the ribavirine (Virazol), an analogue of guanosine, wich when added to the medium at concentrations of 10-50 mg/l, was effective against PVX, PVY, PVS and PVM in potato [2, 3, 6, 10, 11]. However, ribavirin at active dose is usually phytotoxic causing an increase in culture time, death of some meristems, and the need for frequent transfers to fresh media [10]. In our research we used oseltamivir (Tamiflu) for reducing the phytotoxic effect of the ribavirine.

The methods employed to eliminate viruses from plants like meristem culture, chemotherapy and thermotherapy are technically demanding and time consuming. Electrotherapy, however, is a simple method of virus eradication without the need to use any special or expensive equipment. In this technique, the electric current is applied to

plant tissues in order to disrupt or degrade viral nucleoprotein and eliminate its virulence activity [7,12,13].

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MATERIAL AND METHOD

Solanum tuberosum L. microplants cv. Roclas, tested virus free, were obtained from the Biotechnology Department of National Institute of Research and Development for Potato and Sugar Beet Brasov. The microplants were transferred to greenhouse conditions 30 days. For obtaining positive material, a part of these plants were mechanically inoculated [1] using secondary infected sources (two isolates for each virus). The plants had previously tested positive by ELISA for PVY and PVX respectively, to confirm the occurrence of single infection by PVY or by PVX in the selected material. Tissue samples infected mother plants growing in the greenhouse were used as positive control. Stem segments excised from infected potato plants were transferred two times in MS medium with antiviral compounds (sub-culture S1 – 26 days, sub-culture S2 – 30 days). Plantlets obtained were divided into single node cuttings (about 1cm length) and sub-cultured on a fresh MS medium (sub-culture S3). After 28 days the plantlets were planted in pots, under greenhouse conditions.

DAS ELISA test. The antiserum and conjugated used for viruses detection were obtained in our laboratory [5]. The analysis was performed following the protocol described by Clark and Adams [4] (100 μl per well).

Chemotherapy was carried out on nodal cuttings with a single axillary bud and was undertaken with ribavirin (RBV, Sigma, Q0125) and oseltamivir (OSMV, Tamiflu, LaRoche) in the following way: RBV 40 $mg \cdot l^{-1}$ + OSMV 40 $mg \cdot l^{-1}$.

Electrotherapy treatments and regeneration of virus-free plants. Before the treatment, the greenhouse-grown inoculated plants were assayed by DAS-ELISA for verify the virus presence. Plants with similar levels of virus concentration were used to obtain stem segments containing axillary buds for electrotherapy. Each infected plant provided for approximately 3 nodal cuttings that were subsequently used for electrotherapy treatment. From each stem one node was cut for the control (untreated by electrotherapy) and the stem segments remaining were immersed in natrium chloride solution (1M) in an electrophoresis tank and exposed to electric currents of 100 mA for 5, 10 and 20 minutes using a power supply (Tehsys E250V). After treatment, the stems were surface sterilized using 96% ethanol for 30 s followed by 0.1% sodium hypoclorite for 1 min and rinsed three times in distilled water. Explants were prepared by dividing stem segments into nodal cuttings with a single axillary bud. The cuttings were cultured in test tubes containing MS medium with antivirals compounds.

In the aime to estimate an electrotherapy treatment leading to high rates of both virus elimination and plant regeneration, the Therapy Efficiency Index (TEI) was used [12]. The TEI was estimated with the following relation:

TEI = percentage of regenerated plantlets × percentage of virus-free samples / 100

Statistical analysis. Data were analyzed by ANOVA and Duncan's Multiple Range Test and scored as significant if $P<0.05$.

RESULTS AND DISCUSSIONS

Application of electrotherapy on the potato cultivar 'Roclas' resulted in successful elimination of PVX from potato tissues when the most severe treatments were applied (100 mA for 10-20 minutes).

The Figure 1 showed that the two viruses were not very different in responding to the treatments (excepting the variant 100mA, 10minutes). The success of electrotherapy in producing virus-free plants depends upon both plant regeneration and virus elimination rates. An increase in the number of virus-free plants for both viruses was observed as the intensity of the electric current was raised.

After the third subculture (S3), the mean values for regeneration rate (estimated as percentage of total number of regenerated plants from cultivated plants) was 47,6-61,9% for the material PVY infected and treated, respectively 55,0- 72.22% for PVX infected explants treated by electrotherapy and chemotherapy. Concerning the PVY infected material, the action of current applied 5, 10, 20 minutes, lead to the following values of the regeneration rate 51,3%, 61,9% and 47,6% (Table 1). The plants PVX infected had a different behavior, the regeneration rate (the mean values for all the variants) recorded to different values in the variants, in function of the time period of electrotherapy treatment. Thus, in case of material PVX initially infected, using three durations of electrical current (5, 10, 20minutes) the following values were obtained for the values of regeneration rate mean: 63,6%, 72,22% and 55,0% (Table 1). The values obtained for regeneration rate of infected material with PVX were higher than those specified in bibliography [10].

Table 1

Mean values for regeneration rate of the PVY and PVX plants treated (cv. Roclas)

Variant	Electro therapy mA/min	The rate regeneration* of infected material			
		PVY		PVX	
		Regenerated plants[a]/ Treated plants[b]	%±SD	Regenerated plants[a]/ Treated plants[b b]	%±SD
Positive control	0/0	3/30	10.0±2.431	5/28	187.8±3.521
Negative control	0/0	10/12	83.3±2.944	12/14	85.7±6.834
V2	0/0	18/42	42,7±8.611	20/42	47.6±10.672
V7	100/5	20/39	51.3±1.852	28/44	63.6±7.157
V8	100/10	26/42	61.9±9.659	26/36	72.22±9.81
V9	100/20	20/42	47.6±8.392	22/40	55.0±4.064

[a] number of regenerated plants (all repetitions);
[b] number of treated explants (all repetitions);
*Values = mean of three repetitions. The results were recorded after three subcultures: 2 subcultures S1, S2 explants treated by electrotherapy and a subculture S3 untreated explants transferred on medium MS. S1=subculture on medium MS with antivirals (Oseltamivir 40mg/l +Ribavirin 40mg/l).
Abreviations: PVY= potato virus Y; PVX= potato virus X; SD= standard deviation

The most severe treatment affected the regeneration rate values. Even in these conditions, despite the low percentage of this indicator, the higher values for TEI (Figure 1) were obtained in the variants with the higher treatment time (100 mA, 10 minutes, respectively 20 minute).

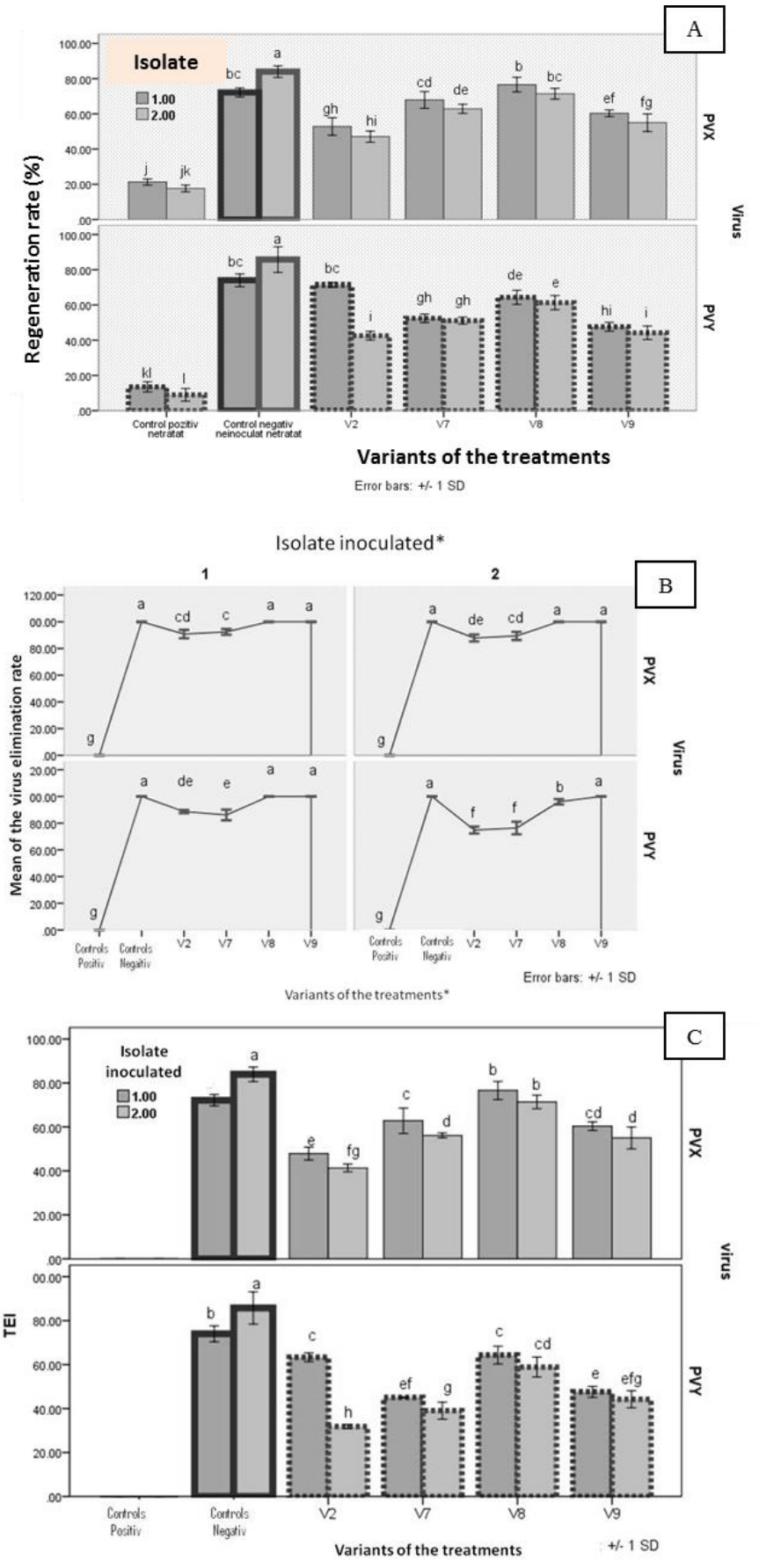

Figure 1. Effects of treatments on regeneration rate (A), virus elimination rate (B) and Therapy Efficiency Index-TEI (C) of the tested material (cv. Roclas PVX and PVY infected and treated).

All the plants regenerated from variant V8, after electrotherapy and transferred on medium with antivirals were viruses free for the material PVX infected and in case of plants PVY infected, only 94% from plants were virus free. For the variants V7 the *virus elimination* rate was 78.00% for material inoculated with PVY and treated, respectively 90.22% for material inoculated with PVX.The highest virus elimination rate was obtained at the highest time period of the treatment with electric current (20min, 100mA). Although raising the treatment time, increased the mean virus elimination rate, it also decreased the mean plant regeneration rate, so TEI index has to be used as a basis in identifying the most efficient electrotherapy treatment.

The results of the present research work show that the regeneration of explants *in vitro* is influenced by electrotherapy treatment and depends upon the electric current intensity. Many papers suggest that the regeneration rate of virus-free plants obtained after electrotherapy is higher than that of plants exposed to more conventional virus elimination techniques including meristem culture and chemotherapy [14,15].

Usually, plant regeneration depends upon several factors, including genotype, physiological state of the explant, culture medium, the cultivation conditions and the interactions between these factors [17]. The electric pulses are also reported as stimulants of plant differentiation *in vitro* [7]. It was demonstrated that regeneration of potato plant tissues could be improved by exposing explants to mild electric currents [12].

Sometime the electrotherapy technique is not more efficient than other conventional techniques in eliminating viruses from plant tissues. However, it seemed to be more effective, faster, easier and less demanding than other methodes in regenerating virus-free plants. It can also be effectively combined with chemotherapy as demonstrated earlier [14,15]. It has been postulated as a hypothesis that viral nucleoproteins may be denatured by when it is exposed to electric current [12]. It has been suggested that denaturation of viral particles may occur during transport through the plasmodesmata in the apoplastic space. Inactivation of specific nucleoprotein that assist in cell-to-cell movement to threedimensional structures leads to blockage, which prevents further penetration of virus particles to healthy cells [7,13]. The molecular basis of this phenomen is poorly understood.

CONCLUSIONS

This study revealed that chemotherapy (40 mg/l RBV + 40 mg/l OSMV) and electrotherapy (100mA, 10 minutes), had beneficial effects on PVX and PVY elimination from potato plant tissues. But, some elements remain to be tested and/or improved:

- the treatments success is cultivar dependent (we used only cv.'Roclas')
- the phytotoxicity of the treatments has to be verify
- to estimate the efficiency of the treatments with bulked samples is required

Further investigations are needed for combine chemotherapy + electrotherapy, for improvement and optimization of these techniques.

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