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Effects of some electrotherapy treatments of pvx infected potato plantlets cv. Roclas, on several biological development indicators

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Abstract The purpose of this study was to estimate several toxicity effects of some treatments (electrotherapy in tissue culture) used for decrease PVX (potato virus X) infection level. The biological material used in experiments was plants (variety Roclas, virus free biological material) mechanically inoculated using PVX secondary infected plants from Ostara variety. Electrotherapy was applied in 9 variants: after washing and sizing explants, potato stems infected were exposed to either 40, 50 or 100 miliampers (mA), for 5, 10 or 20 minutes, followed by sterilization and planting the axillary buds tip in vitro. The biological indicators estimated were the following: multiplication rate, mean leaf number and mean long stem of the treated material. The electrotherapy variant 10minutes at 100mA showed the maximum values of the multiplication rate in all subcultures for all the PVX infected material. This treatments variant had positive effects on the other biological indicators estimated by biometric measurements of the material obtained from treated vitro-plants.

Key words

potato virus X, multiplication rate, electrotherapy

Potato virus X (PVX), a *Potexvirus*, is a dangerous pathogen for potato crop because it occurs throughout commercial stocks of most varieties and is responsible for many of the uncertainties and difficulties encountered in field inspections. When *potato virus Y* is present, synergy between these two viruses causes severe symptoms in potatoes [1].

Elimination of PVX from potato crop is essential for seed potato production. Also, in many studies, the efficiency of some techniques (chemotherapy, electrotherapy) in eliminating PVX and producing virus-free plants (cultivar Roclas) was evaluated [3, 4]. But it is very important to know the effects of these treatments on the plants development [1].

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 [5, 8, 11]. 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 methods in regenerating virus-free plants. It can also be effectively combined with chemotherapy as demonstrated earlier [7, 9].

It has been postulated as a hypothesis that viral nucleoproteins may be denatured by when it is exposed to electric current [8]. 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 three dimensional structures leads to blockage, which prevents further penetration of virus particles to healthy cells [5, 6]. The basis of this observation is still poorly understood.
The study aimed to evaluate the phyto toxicity effects of several electrotherapy treatments used for PVX elimination in potato plants and find out the best one both for virus eradication and for an optimal next plants development.

Material and Method

Biological material. *Solanum tuberosum L.* vitro plants 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 vitro plants were transferred to greenhouse conditions 30 days. For obtaining positive material, a part of these plants were mechanically inoculated [1] using a PVX secondary infected source variety Ostara. The plants had previously tested positive by ELISA for PVX, to confirm the occurrence of single infection in the selected material. Plants with similar levels of virus concentration were used to obtain stem segments

containing axillary buds for electrotherapy. Tissue samples infected mother plants growing in the greenhouse were used as positive control.

Electrotherapy treatments and regeneration of virus-free plants. 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 40, 50 and 100 mA for 5, 10 and 20 minutes using a power supply (Tehsys E250V, fig. 1, table 1) [1]. After treatment, the stems were surface sterilized and rinsed three times in distilled water (fig. 1). Explants were prepared by dividing stem segments into nodal cuttings with a single axillary bud. The cuttings were cultured in test tubes containing Murashige (MS) medium [10] (fig. 1). The experiment was repeated three times for each electrotherapy treatment. Stem segments excised from infected potato plants were transferred three times in MS medium (sub-culture S1-26 days, sub-culture S2 -30 days, sub-culture S3-28 days). Only before the subcultures S1 and S2 the material was treated by electrotherapy. Plantlets obtained in all the subculture were divided into single node cuttings (about 1cm length) and sub-cultured on a fresh MS medium.

Multiplication rate was estimate by count the single nodes for each plant, in all the variants.

DAS ELISA test. The analysis was performed following the protocol Clark and Adams (1977) [2].

Table 1

Variants of the plantlets (cv. Roclas) treatments with electric current (intensity / times)

Variant	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9
Treatment (mA/min)	0/0	40/5	40/10	40/20	50/5	50/10	50/20	100/5	100/10	100/20

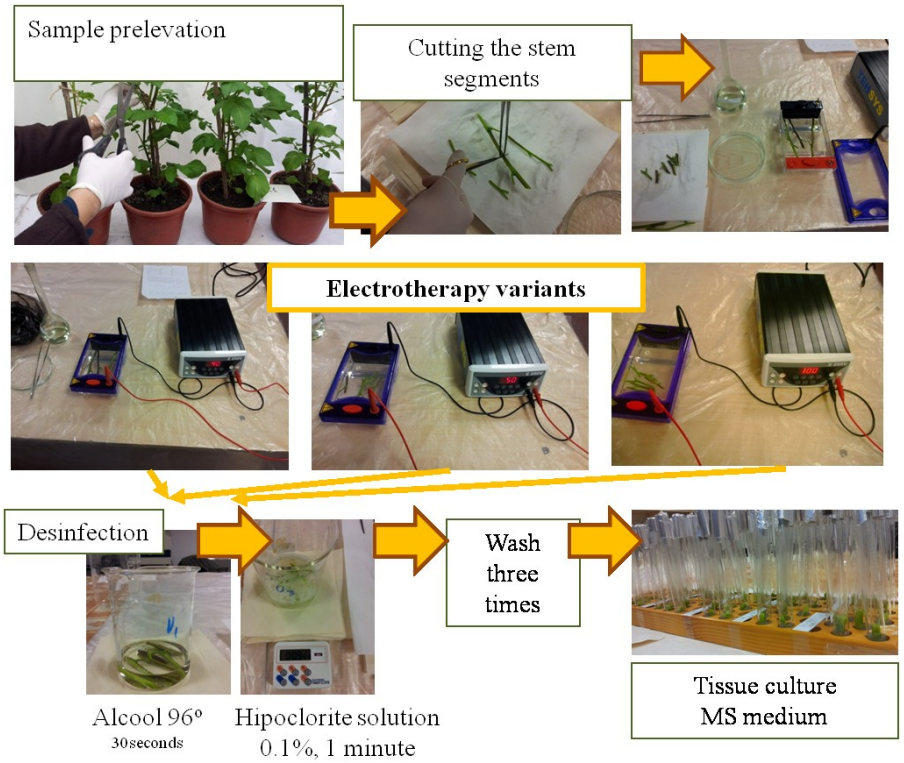

Fig. 1 Electrotherapy treatments steps and equipment used to produce virus-free plants in potato: Power supply and electrophoresis tank used for producing electric currents, single node explants prepared for in vitro culture, electrotherapy variants (intensity of the electric current) of electrotherapy treated plants on MS medium [1].

Results and Discussions

The phytotoxic effect of the electro therapies (several variants of electric current intensities, time of the treatment) was estimate by a specific indicator: multiplication rate (number of single nodes steam obtained from the first node propagated). To the second and third subculture were used only two explants of apex.

The experimental results (figure 2) highlight the significant influence, which had the current intensity and duration of treatment on the rate of explants *in vitro* multiplication, in case of all the variants. Thereby, it was observed that the multiplication rate increased compared to untreated control, for all variants, benefic effect of electrotherapy being significant in all the subcultures .

The evaluation the rate of multiplication, for variants that has been used the maximum current intensity (100mA) revealed different results, depending on the sub subculture. To material infected with PVX was observed a decrease in multiplication rate, especially to the long duration (20 minutes), particularly at subculture 2, at exposure of material to the electric current 50 and 100 mA (Fig. 2). Maximum values of multiplication rate were observed in variants that used the biggest intensity of the electric current (100mA) (fig. 2)

In the case of plantlets PVX infected, significantly high multiplication rates were recorded at S1 subculture, significantly higher values was observed at variant V9 (100mA/ 20 minutes).

Only at the first subculture (S1), it was observed a significant and progressive increase in the rate of multiplication once with increased the severity of treatments.

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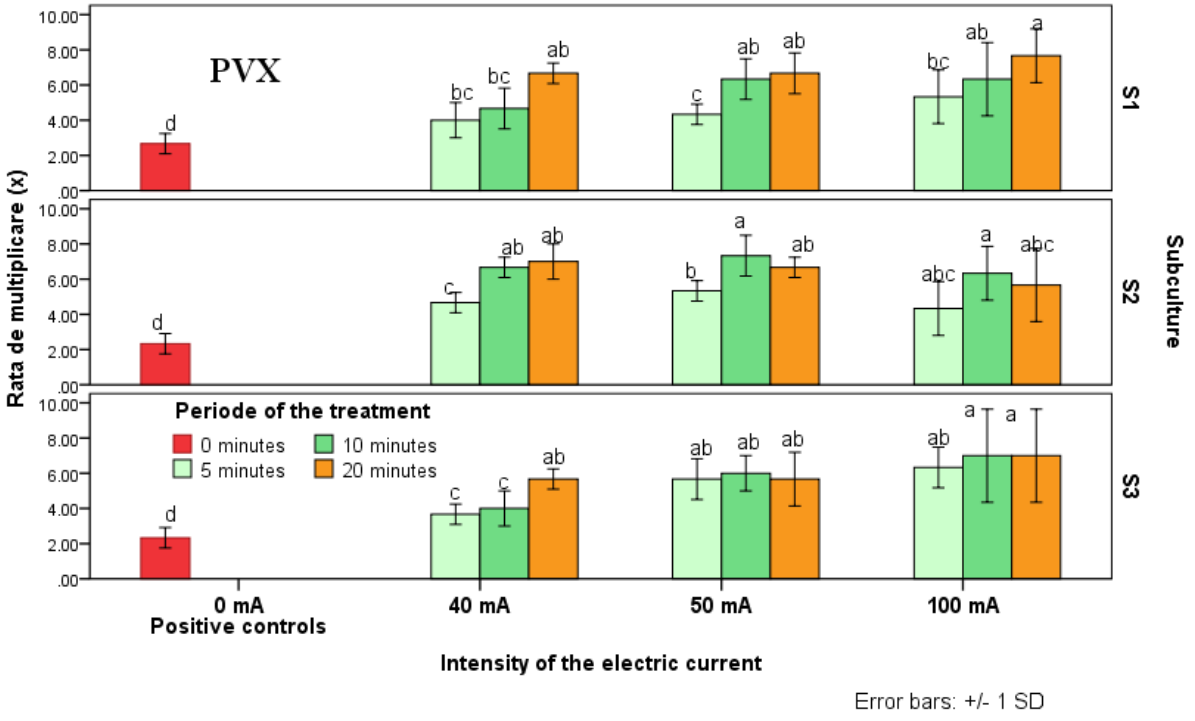

Fig. 2 Effects of the treatments (electric current intensity, times) on the multiplication rate of the material (cv. Roclas) infected with PVX and treated, comparatively with the positive control (infected untreated), in 3 subcultures. The values represent mean of 3 repetitions. Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test.

Biometric measurements of the potato plantlets submitted to electrotherapy pursued estimation of electric current (especially in the case of the most severe variations of 100 mA / 10-20 minutes) over development of explants, in order to choose the optimal variant, allowing not only the elimination of viral infection, and optimum evolution of material in vegetation, plants survival.

These measurements aimed estimating the treatments effect over:

- the number of leaves / plantlets
- the stem plantlets length.

Measurements were made during subcultures only for vitro plants that were the starting point for further subculture. Were chosen each time from each variant plantlets, that developed the most harmonious.
The obtained results exemplified graphically for PVX infected and treated vitroplants highlights the strong effect of electrotherapy treatments over number of leaves and length of the stem, for all plantlets measured as compared to the positive control (infected and untreated).

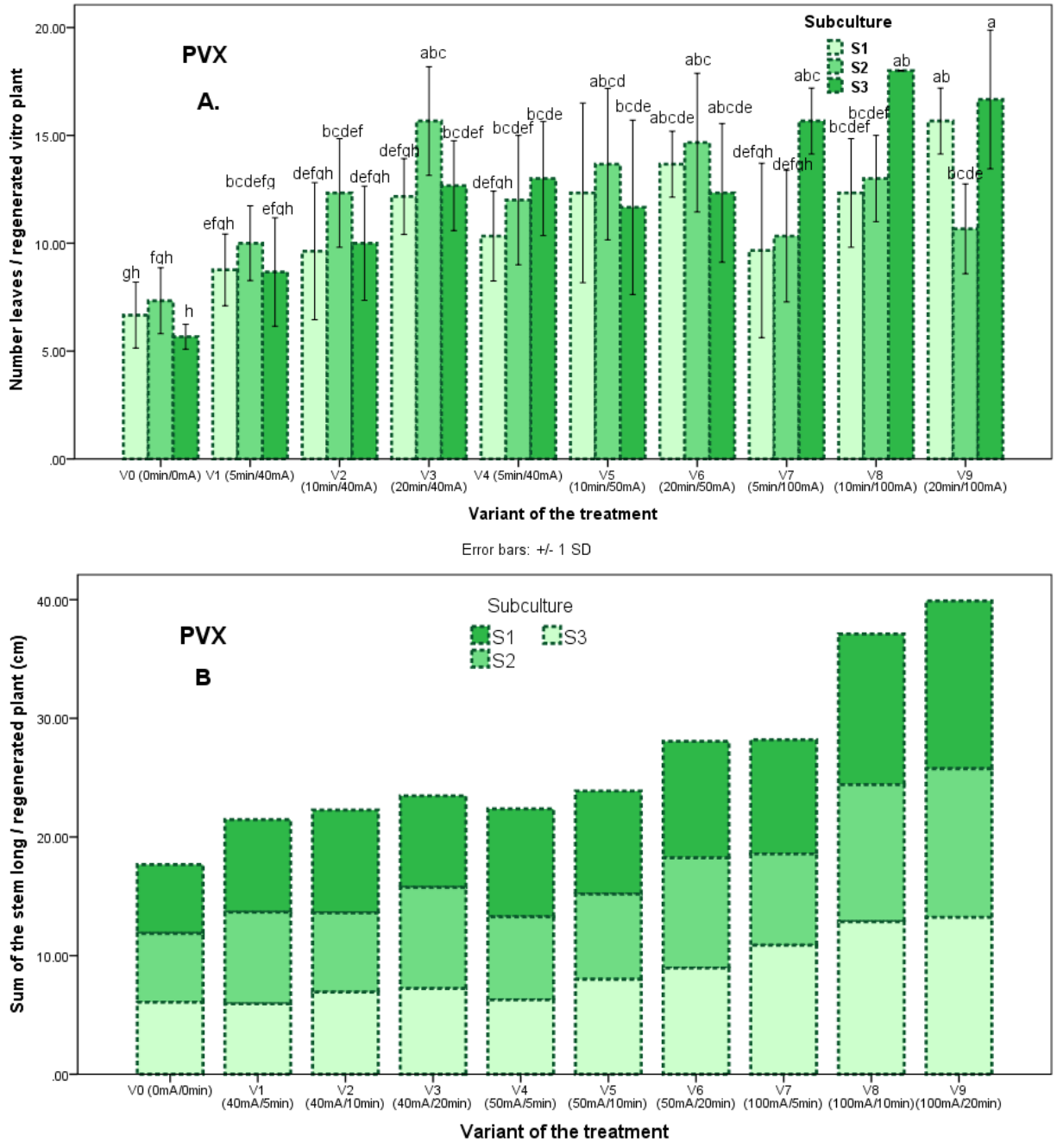

Fig. 3 Estimation of the effect of electrotherapy treatment on the development of the material (cv. Roclas) infected with PVX and treated, comparatively with the positive control (infected untreated), in 3 subcultures S1, S2, S3 by biometric measurements (A) Number of leaves/ regenerated plant. (B) Sum of the stem long / regenerated plant. Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test

The biggest differences compared with to untreated control, were recorded for the variants of treatment more severe, both for the average number of leaves/plantlet, and for the mean values of the stem /plantlet.

Application of electrotherapy on the potato cultivar Roclas resulted in partial elimination of PVX from potato tissues when the most severe treatments were applied (100 mA for 10-20 minutes) [1]. The figure 3 showed that the biological indicators were not very different in responding to electrotherapy applying to PVX infected material (excepting the variant 100mA, 10minutes). In spite of developing of many virus-free plants [1], increasing levels of biological indicator values were observed in all variants even if the regenerated plantlets remain infected [1]. The success of electrotherapy in producing virus-free plants depends upon both plant multiplication rates, upon the next development of the plants. Usually, this indicator depends upon several factors, including genotype, physiological state of the explant, culture medium, the cultivation conditions and the interactions between these factors [12]. The electric pulses are also reported as stimulants of plant differentiation *in vitro* [5]. It was demonstrated that regeneration of potato plant tissues could be improved by exposing explants to mild electric currents [8].

The results of the present research work show that the multiplication rate of explants *in vitro* is influenced by electrotherapy treatment and depends upon the electric current intensity. Many papers suggest that the multiplication rate of virus-free plants obtained after electrotherapy is higher than that of plants exposed to more conventional virus elimination techniques including in vitro tissues culture and chemotherapy [7, 9]. In our study we obtained good results regarding the multiplication rate when higher intensities of the electric current was used (100mA/10minutes).

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Conclusions

This preliminary study revealed that the electrotherapy (100mA, 10 minutes) of PVX infected material had beneficial effects on the multiplication rate of the plants. Further investigations are needed for improvement and optimization of these techniques, because it is possible to obtain virus free material using electrotherapy treatment [1].

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