THE EFFECT OF SOME THERAPIES ON POTATO VIRUS Y AND POTATO VIRUS X INFECTED *Solanum tuberosum* L. PLANTLETS (cv. 'Roclas')

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Abstract. The purpose of this study is to decrease the PVY (potato virus Y) and PVX (potato virus X) infection level, using electrotherapies, antiviral compounds (ribavirin and oseltamivir) in the tissue culture and several treatments (*Satureja hortensis* essential oils, H_2O_2 1mM pH 5.6 and AA 3mM pH 5.6) applied by spraying the microplants acclimatized in greenhouse. The biological material used was plants (variety 'Roclas', virus free biological material) mechanically inoculated using: PVY secondary infected plants from 'Record' variety; PVX secondary infected plants from 'Bintje' 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 immediate planting the axillary buds tip *in vitro*. Chemotherapy was undertaken with ribavirin (RBV) and oseltamivir (OSMV) (RBV 40 $mg \cdot l^{-1}$ + OSMV 40 $mg \cdot l^{-1}$; RBV 20 $mg \cdot l^{-1}$ + OSMV 40 $mg \cdot l^{-1}$; RBV 20 $mg \cdot l^{-1}$ + OSMV 80$mg \cdot l^{-1}$). The first variant (RBV 40 $mg \cdot l^{-1}$ + OSMV 40 $mg \cdot l^{-1}$ added to the tissue culture medium + essential oils treatments of acclimatized plants) and the electrotherapy variant 10 minutes at 100mA showe the highest rate of virus eradication, the maximum values of the therapy efficiency.

Keywords: *Satureja hortensis* essential oils; PVY; PVX; ribavirin; oseltamivir; electrotherapy.

Abbreviations EOs essential oils; AO antioxidants; PVY potato virus Y; PVX potato virus X; RBV ribavirin; OSMV oseltamivir; MS Murashige Skoog; AA ascorbic acid; S sub-culture; TEI therapy efficiency index

INTRODUCTION

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 [24].

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.

Elimination of PVY and PVX from potato supply is essential for seed potato production. Also, in this study, the efficiency of some techniques (chemotherapy, treatments with *Satureja hortensis* oils and antioxidants, 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. The most used substance is the ribavirin (Virazol), an analogue of guanosine [23], wich when added to the medium at concentrations of 10-50 mg/l, was effective against PVX, PVY, PVS and PVM in potato [3, 4, 7, 8, 15, 16]. 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 [15]. Regeneration of potato sprouts from meristematic cultures was delayed by 6 to 8 weeks [4]. To overcome toxicity, a low dose of ribavirin was supplied with another compounds (antimethabolites). The simultan use of the two chemicals alone was also beneficial, because ribavirin above 5 mg/l delayed meristem development. In our research we used oseltamivir (Tamiflu) for reducing the phytotoxic effect of the ribavirin.

The treatments with *Satureja hortensis* essential oils and antioxidants (H_2O_2 and ascorbic acid) applied to acclimatised plants (obtained by transfering the plantlets) could be beneficial for obtaining virus free material. The essential oils from *Satureja hortensis* L. (summer savory – Family *Lamiaceae*, order *Lamiales*) are known for its antiseptic (antifungal and antiviral) properties [2]. Maybe, some compounds of these oils could be implicated in the processus signaling against stress, in infected potato plants [1, 2]. Plant cells have defensive responses to pathogen attack associated with changes in oxidative metabolism [11]. H_2O_2 is believed to play two distinct roles in pathogenesis: one involves the oxidative burst in the hypersensitive response, which restricts pathogen growth [17, 18] and the other activates plant defense responses (induction of phytoalexins, second messengers - mediating the acquisition of tolerance to both biotic and abiotic stresses and providing information about changes in the external environment) or signaling intermediates and antioxidant enzymes [17].

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 [12].

The use of electric current for the production of virus-free plants has been reported for a number of crop plants since the 1990's. This technique was first employed for the elimination of PVX from potato plants in which an electric current of 15 mA for 5 minutes led to 60–100% elimination of this virus in various cultivars [19]. Electrotherapy has also been used for elimination of *Potato virus Y* (PVY), *Potato virus A* (PVA), *Potato virus S* (PVS) and *Potato leaf roll virus* (PLRV) [12, 22]. In addition, to another plants (*Vitis vinifera* L.) the genetic stability, fidelity and uniformity of plants regenerated through electrotherapy have not been affected as evaluated morphologically or molecularly by DNA markers [9].

This research started because high yielding commercial varieties shown durable resistance against potato viruses (especially for PVY and PVX) in Romania are not available. The study aimed to evaluate the therapy efficiency of different chemotherapies and treatments with *S. hortensis* EOs + AO applied to acclimatised plants and several electrotherapies, for PVY and PVX elimination in potato plants and find out the best one for virus eradication.

MATERIALS AND METHODS

Plants material. *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. Single node cuttings were propagated in test tubes on Murashige and Skoog medium [21] at 20±1°C under a 16 h photoperiod. The microplants were transferred to greenhouse conditions 30 days after the single-node subculture step. For obtaining positive material, a part of these plants were mechanically inoculated [1] using a PVY secondary infected source cv. Record and respectively a PVX secondary infected source cv. Ostara.

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.

Acclimatization, treatments with *Satureja hortensis* **essential oils and antioxidants**

Solanum tuberosum L. plantlets submitted to chemotherapy, regenerated with roots and a well developed aerial part (5-7 leaflets), were removed from the culture medium and were acclimated in pots and kept under a transparent cover. After 7 days for beginning the acclimatization, the plants (excepting the controls) were sprayed twice a week with a *Satureja hortensis* essential oils suspension (1/1000, 5 ml each plant) and weekly with H_2O_2 (1mM pH 5.6) and AA (3mM pH 5.6) [1]. Weekly, we applied only one of the H_2O_2 (1mM pH 5.6) and AA (3mM pH 5.6) solutions: the week when we applied H_2O_2, we didn't used ascorbic acid. The controls were sprayed with distilled water. The survivor plants were indexed after 45 days.

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 [6] (100 µl per well). Microplates were filled with substrate solution (p-nitro phenyl phosphate) incubated 1 hour for PVY and 30 minutes for PVX, and the absorbance values were estimated at 405 nm (A_{405}) on Tecan reader (softwere Magellan). The samples having A_{405} values exceeding two times the average of healthy controls were considered virus infected.

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 variants: V1= RBV 20 $mg \cdot l^{-1}$ + OSMV 40 $mg \cdot l^{-1}$; V2 = RBV 40 $mg \cdot l^{-1}$ + OSMV 40 $mg \cdot l^{-1}$; V3 = RBV 20 $mg \cdot l^{-1}$ + OSMV 80 $mg \cdot l^{-1}$.

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 40, 50 and 100 mA for 5, 10 and 20 minutes using a power supply (Tehsys E250V, fig. 1). 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 (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 MS medium (fig. 1). The experiment was repeated three times for each electrotherapy treatment.

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 [19]. The TEI was estimated with the following relation:

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

Table 1. Chemicals used for obtaining PVY and PVX virus-free plantlets

Chemicals	Activities	Chemicals Activities References
Ribavirin (Virazol) (1,ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide) (Abbreviation in text RBV)	Broad spectrum anti-viral activities, ribavirin 5'-phosphate: inhibitor of inosine monophosphate (IMP) dehydrogenase	Cassel and Long (1982) [3]
Oseltamivir (Tamiflu) [ethyl (3R,4R,5S)-5-amino-4-acetamido-3-(pentan-3-yloxy)-cyclohex-1-ene-1-carboxylate] (Abbreviation in text OSMV)	- an antiviral prodrug - used to slow the spread of flu virus (influenza A and B) by stopping from chemically cutting with its host cell. - produced from shikimic acid, an inhibitor of neuraminidase	Ward et al. (2005) [26]

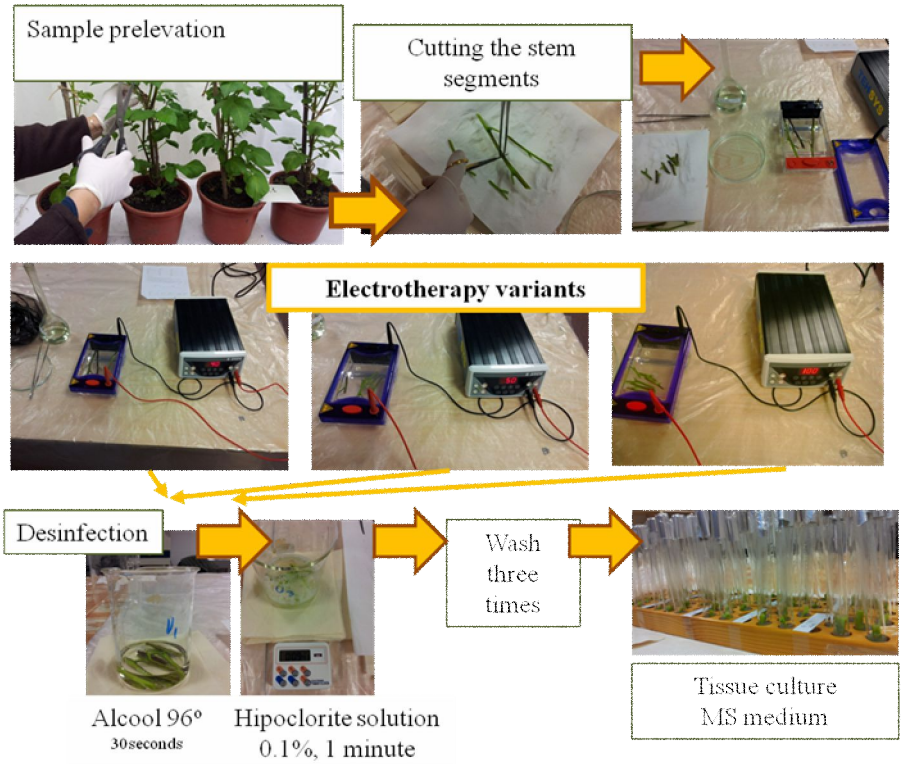


Figure 1. Electrotherapy treatments steps and equipment used to produce virus-free plants in potato cv. 'Roclas': Power supply and electrophoresis tank used for producing electric currents, single node explants prepared for *in vitro* culture, electrotherapy variants (intensity of the electric current)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

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

RESULTS

Chemotherapy

Results showed that in all the variants and stage of the therapy, for both viruses, plants virus free were found by DAS ELISA (tables 2, 3).

Regarding the PVX infected plants, the chemotherapy variant V2 (RBV 40 mg/l + OSMV 40 mg/l) combined with treatments with EOs and AO of acclimatized plants, lead to the higher value for the virus elimination rate (100% PVX free plants) and for the therapy efficiency index (TEI): 87.5%. (table 2; fig. 2A). Highest values for the virus elimination rate (100%) were obtained in variant V3 (RBV 20 mg/l + OSMV 80 mg/l) combined with treatments *EOs* +AO too, but this treatments decreased regeneration rate (50%), also the TEI had lower values than in variant V2 (table 2; fig. 2B).The absorbance values at 405nm (DAS ELISA) for the plants acclimatized obtained from the variant V2 (RBV 40 mg/l + OSMV 40 mg/l) and treated wit EOs and AO were significantly lower (fig. 2B).

For the PVY infected plants, the chemotherapy variant V2 (RBV 40 mg/l + OSMV 40 mg/l) combined with treatments with *S. hortensis* essential oils and ascorbic acid (3mM) or H_2O_2 (1mM) of acclimatized plants was the best again, leading to higher value for the virus elimination rate (83.3% PVY free plants) and for the highest therapy efficiency index (TEI): 62.5%. (table 3; fig. 3A). The highest values for the virus elimination rate (100%) were obtained in variant V3 (RBV 20 mg/l + OSMV 80 mg/l) combined with treatments EOs +AA (3mM)/ H_2O_2 (1mM), but this treatment decreased regeneration rate (57.0% comparated with that obtained for variant V2 75%), also the TEI had lower values than in variant V2 (table 3; fig. 3A).

The absorbance values (DAS ELISA, 405nm) for the plants acclimatized obtained from the variant V2 (RBV 40 mg/l + OSMV 40 mg/l) and for variant V3

Table 2. Efficiency of chemotherapy + treatments* of acclimatized plants on PVX infected material regeneration and virus eradication (cv. 'Roclas')

Variant of the treatment (for potato virus X infected plants)		Regeneration rate		Virus elimination rate	
		NTP/NRP	%	NPVF/NTP	%
V1	S1 (MS + antivirals)	5/6	83.3	1/5	40
	S2 (MS + antivirals)	10/12	83.3	5/10	50
	S3 (MS)	16/18	88.9	8/16	***50.0***
	Plants acclimatized untreated	6/6	100	3/6	50
	Plants acclimatized treated *	5/6	**83.3**	3/5	**60**
V2	S1 (MS + antivirals)	7/8	87.5	5/7	71.4
	S2 (MS + antivirals)	12/14	85.7	10/12	83.3
	S3 (MS)	22/24	91.7	18/22	***81.8***
	Plants acclimatized untreated	7/8	87.5	5/7	71.4
	Plants acclimatized treated *	7/8	**87.5**	7/7	**100**
V3	S1 (MS + antivirals)	5/8	62.5	4/5	80
	S2 (MS + antivirals)	7/10	70.0	6/7	87.5
	S3 (MS)	10/16	62.5	9/10	***90***
	Plants acclimatized untreated	4/6	66.7	3/4	66.67
	Plants acclimatized treated *	3/6	**50.0**	3/3	**100**

V1= MS +RBV(20 mg/L) + OSMV(40 mg/L); V2 = MS +RBV(40 mg/L) + OSMV(40 mg/L);V3= MS +RBV(20 mg/L) + OSMV(80 mg/L); S1-S3= sub-cultures 1-3; MS =Murashige and Skoog medium; RBV=ribavirin; OSMV=oseltamivir; NTP = number of tested plants (plants that survived); NRP = number regenerated plants; NPVF = number of plants virus free;
*Treatments with *Satureja hortensis* essential oils and ascorbic acid (3mM) and H_2O_2 (1mM)

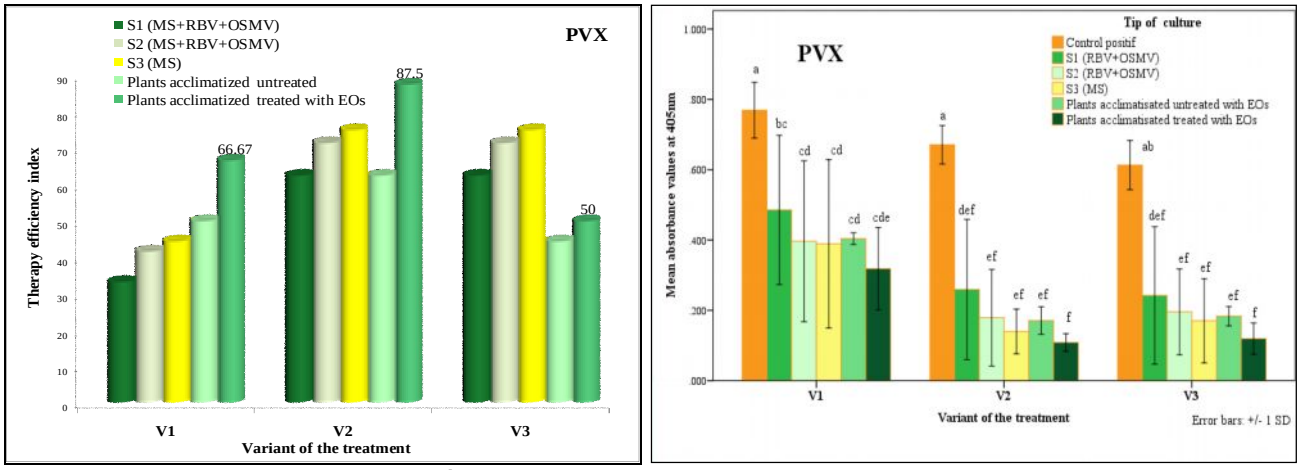


Figure 2. Evaluation of chemotherapy and treatments with *S. hortensis* essential oils and and ascorbic acid (3mM) or H_2O_2 (1mM) at plants infected with potato virus X (PVX).The therapy efficiency of differents variants and vegetation culture (B) The average values of absorbance in the plants regenerated from infected plants inoculated with a PVX viral strains (from cv Bintje secondary infection) Data are means ± SD of 3 experiments (n=3). Bars with different letters differ significantly by ANOVA and Duncan's test ($P<0.05$).

(RBV 20 mg/l + OSMV 80 mg/l) and treated wit *S. hortensis* EOs and hydrogen peroxide (1mM) and ascorbic acid (3mM) were significantly lower (fig. 3B). Chemothetapy applied for PVY infected plants was less efficiently than in case of the biological material infected with PVX, in all the variants. Interesantly was the effect of the treatments applied to acclimatized plants in all variants, these improving the therapy efficiency (table 2, 3; fig. 2A, 3A). This effect was more intensif on the TEI for the material infected with PVY.

Thus, *S. hortensis* EOs and hydrogen peroxide (1mM) and ascorbic acid (3mM) treatments of acclimatizated plants increase the therapy efficiency index (TEI) in all the variants, having beneficial effects on the plants obtained by chemotherapy from PVY and PVX infected potato plants.

Electrotherapy

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).

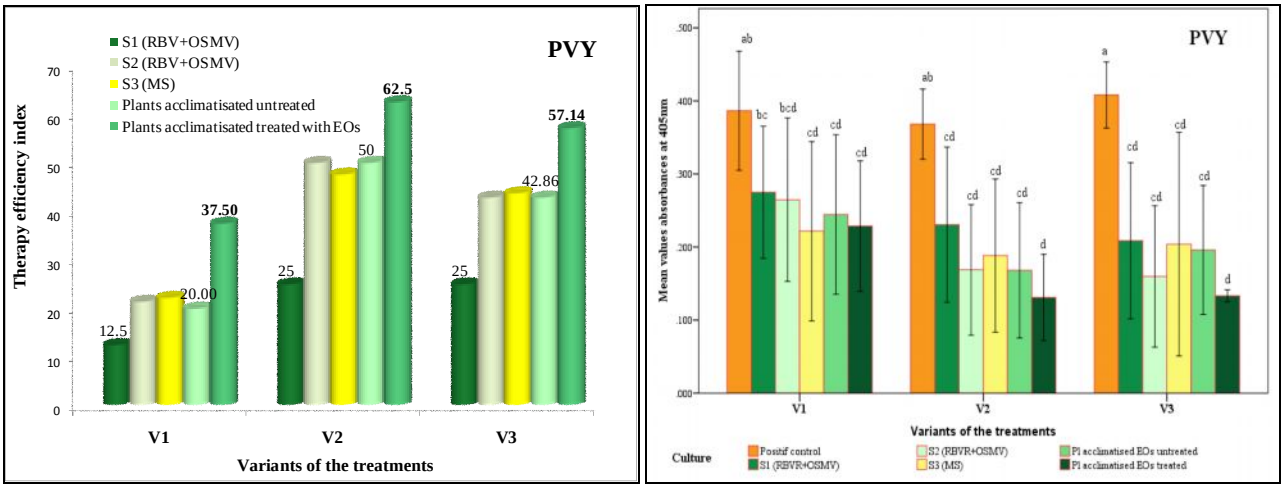
The figures 4, 5 showed that the two viruses were not very different in responding to electrotherapy (excepting the variant 100mA, 10minutes). In spite of developing of many virus-free plants, diminishing levels of virus concentration were observed in all variants even if the regenerated plantlets remain infected, for both viruses (fig. 5). But the success of electrotherapy in producing virus-free plants depends upon both plant regeneration and virus elimination rates.

Plant regeneration rate estimated as the ratio of the number of regenerated plantlets to the total number of

Table 3. Efficiency of chemotherapy + treatments* of acclimatized plants on PVY infected material regeneration and virus eradication (cv.'Roclas')

Variant of the treatment (for potato virus Y infected plants)		Regeneration rate		Virus elimination rate	
		NTP/NRP	%	NPVF/NTP	%
V1	S1 (MS + antivirals)	7/8	87.5	1/7	14.3
	S2 (MS + antivirals)	11/14	78.6	3/11	27.3
	S3 (MS)	13/18	72.2	4/13	30.7
	Plants acclimatized untreated	6/5	60	2/6	33.3
	Plants acclimatized treated*	7/8	**87.5**	3/7	**42.9**
V2	S1 (MS + antivirals)	5/8	62.5	2/5	40.0
	S2 (MS + antivirals)	10/14	71.4	7/10	70.0
	S3 (MS)	16/21	76.2	10/16	62.5
	Plants acclimatized untreated	6/8	75	4/6	66.7
	Plants acclimatized treated *	6/8	**75.0**	5/6	**83.3**
V3	S1 (MS + antivirals)	3/8	37.5	2/3	66.7
	S2 (MS + antivirals)	7/14	50.0	6/7	85.7
	S3 (MS)	9/16	56.2	7/9	77.8
	Plants acclimatized untreated	5/7	71.4	3/5	60.0
	Plants acclimatized treated *	4/7	**57.1**	4/4	**100.0**

V1= MS +RBV(20 mg/L) + OSMV(40 mg/L); V2 = MS +RBV(40 mg/L) + OSMV(40 mg/L);V3= MS +RBV(20 mg/L) + OSMV(80 mg/L); S1, S2, S3= sub-cultures 1,2,3; MS =Murashige and Skoog medium; RBV=ribavirin; OSMV=oseltamivir; NTP = number of tested plants (plants that survived); NRP = number regenerated plants; NPVF = number of plants virus free;
*Treatments with *S. hortensis* essential oils and ascorbic acid (3mM) and H_2O_2 (1mM)



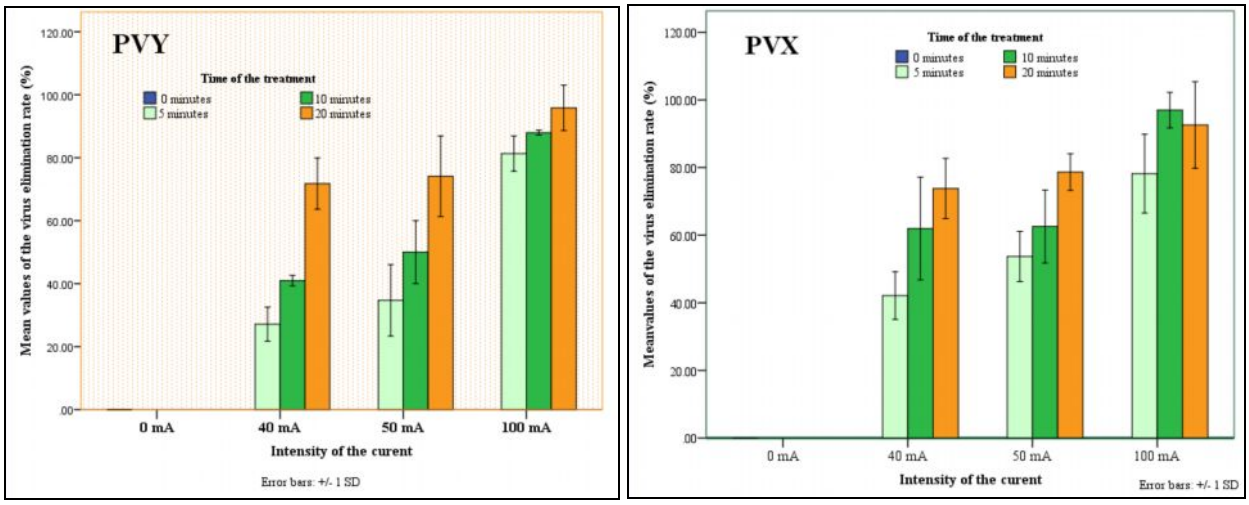
A. **B.**

Figure 3. Evaluation of chemotherapy and treatments with *S. hortensis* essential oils and ascorbic acid (3mM) and H_2O_2 (1mM) at plants infected with potato virus Y (PVY).The therapy efficiency of differents variants and vegetation culture (A) The average values of absorbance in the plants regenerated from infected plants inoculated with a PVY viral strains (from cv Record secondary infection) (B). Data are means ± SD of 3 experiments (n=3). Bars with different letters differ significantly by ANOVA and Duncan's test ($P<0.05$).

cultured explants was 50.0-77.2% for PVY infected and treated plantlets and 56.1- 77.2% for PVX infected material in the cultivar used in this study. The three electric currents of 40, 50, 100mA resulted in 65.6%, 70.0% and 54.0% mean plant regeneration for PVY infected material (table 4). Usualy higher intensities of electric current adversely affected the survival of explants and thus plant regeneration.

Regenerated plantlets were tested for PVX, respectively PVY infection by DAS-ELISA. A reduced concentration of virus was observed in all regenerated plantlets (figure 5). On this basis, the mean virus elimination rates of 'Roclas' cultivar explants exposed different periodes to the three electric currents of 40, 50 and 100mA was 46.6%, 52.6% and 88.3%, for PVY infected explants and 62.7%, 54.9% and 88.7% for PVX infected material respectively (fig. 4). An increase in the number of virus-free plants for both viruses was observed as the intensity of the electric current was raised.

The highest virus elimination rate was obtained at the highest electric current (100mA) used in this study. Although raising the electric current 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 TEI for the three electrotherapy treatments of 40, 50 and 100mA was estimated as 28.6, 36.0 and 47.6 for PVY and 42.6, 44.6 and 64.3 for PVX, respectively. The electric currents of 100 mA for 10 minutes resulted in the highest TEI for both viruses: 52.4 for PVY and 74.3 for PVX (fig. 6).



A. **B.**

Figure 4. Effects of electrotherapy treatments on the elimination rate of PVY (A) and PVX (B) in 'Roclas' cv. infected material. Results are the mean of three experiments.

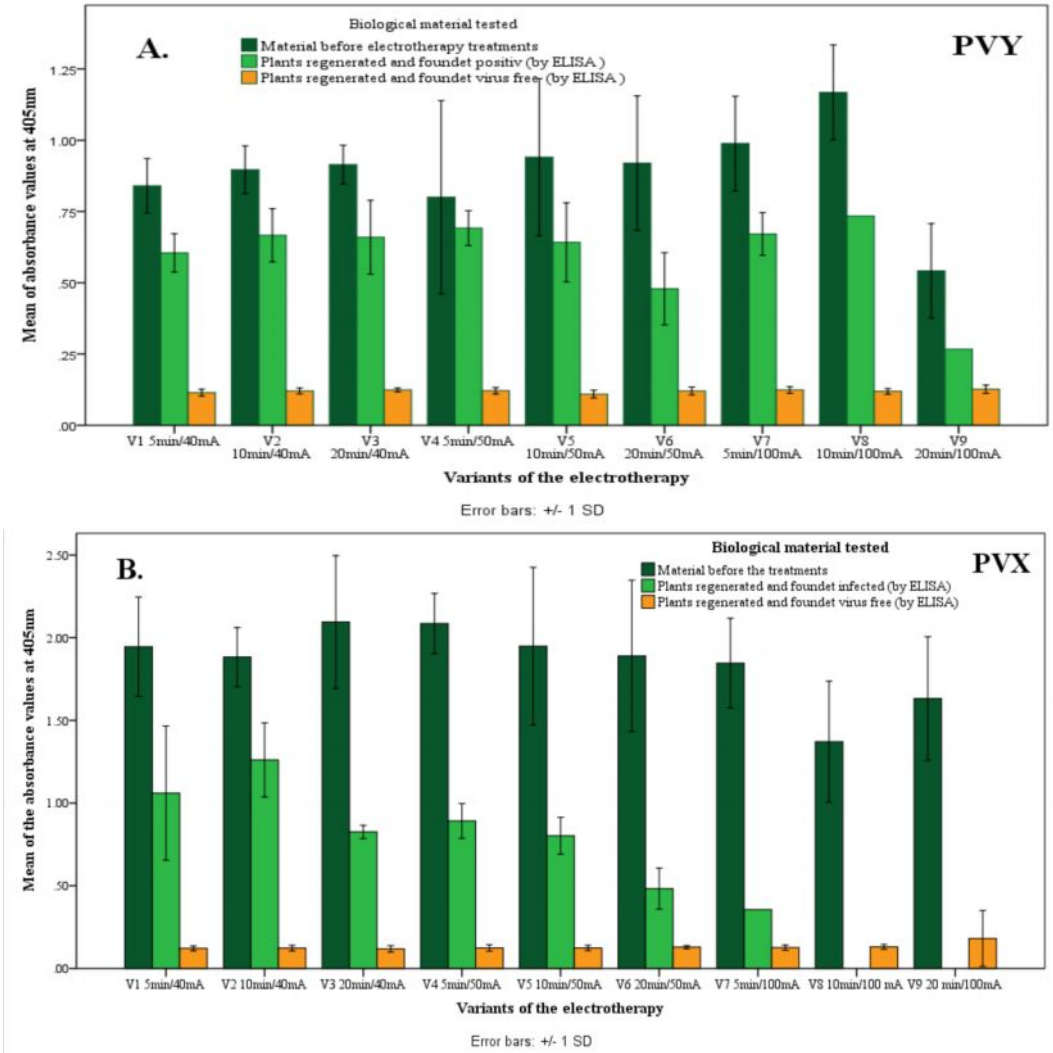
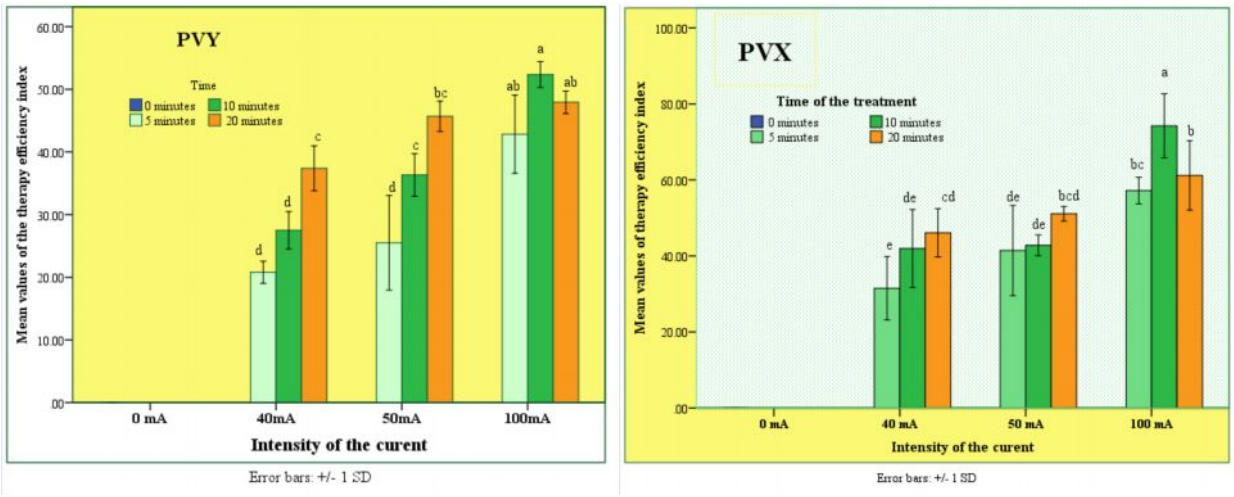


Figure 5. Mean absorbances values of the material tested by ELISA before and after several variants of electrotherapy treatments on potato virus infected plants (cv 'Roclas') with PVY (A) and with PVX (B): values obtained by testing infected plants before electrotherapy (green dark bars), values obtained by testing regenerated plants after electrotherapy which were ELISA positive (green bars) and values obtained by testing regenerated plants after electrotherapy which were ELISA negative (orange bars). Results are the mean of three experiments.

Table 4. Effects of electrotherapy treatments on the regeneration rate for PVY and PVX in 'Roclas' cultivar infected explants. Results are the mean of three experiments

Variant	Treatment mA/min	Regeneration rate of the material infected with:					
		PVY			PVX		
		Regenerated[a]/ treated[b]	%	±STDEV	Regenerated[a]/ treated[b]	%	±STDEV
V0	0/0	5/24	20.8	±4.194	4/21	19.0	± 8.3
V1	40/5	37/48	77.1	±13.38	23/41	56.1	± 11.7
V2	40/10	27/40	67.5	±15.12	26/38	68.4	± 17.1
V3	40/20	25/48	52.1	±13.19	30/48	62.5	± 5.5
V4	50/5	26/35	74.3	±9.311	30/40	75.0	± 12.7
V5	50/10	30/41	73.2	±12.43	24/35	68.6	± 12.3
V6	50/20	30/48	62.5	±2.887	33/51	64.7	± 7.1
V7	100/5	21/40	52.5	±1.925	31/42	73.8	± 6.7
V8	100/10	25/42	59.5	±13.57	27/35	77.2	± 13.0
V9	100/20	24/48	50.0	±12.72	26/39	66.7	± 7.0

[a] number of regenerated plantlets;
[b] number of explants treated
STDEV standard deviation



A. **B.**

Figure 6. Effects of electrotherapy treatments on the 'Roclas' cultivar infected explants with PVY (A) and with PVX (B). Mean of the therapy efficiency index (TEI) for PVY and PVX infected and treated material. Results = mean of three experiments. Bars with different letters differ significantly by ANOVA, Duncan's test (P<0.05).

DISCUSSIONS

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, 20]. In our study we obtained good results regarding the regeneration rate when the oseltamivir was used or when the acclimatized plants were treated with *S. hortensis* EOs and AO.

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 [25]. The electric pulses are also reported as stimulants of plant differentiation *in vitro* [12]. It was demonstrated that regeneration of potato plant tissues could be improved by exposing explants to mild electric currents [19].

According to earlier studies, most species of the *Leguminosae*, with a few exceptions, are difficult to regenerate *in vitro* [10]. The recalcitrance of large-seeded legumes to *in vitro* regeneration could be the result of a long history of inbreeding and selection for high-performing genotypes, which could have lead to a reduction in the genetic variability in modern varieties [10]. Therefore, a plant regeneration rate of 50% to 77.2%, as achieved in this study for PVX, is high compared to the regeneration rate of 9 to 88% obtained by Lozoya-Saldaña et al. 1996 [18]. But regeneration rate in potato is variable and depends on the genetic material.

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, 20]. They showed that nodal cuttings treated by electricity and then cultured in a medium supplemented with

Ribavirin (20 mg/L) resulted in a higher elimination rate of PVY from potato tissues, compared to the cuttings received no ribavirin treatment.

It has been postulated as a hypothesis that viral nucleoproteins may be denatured by when it is exposed to electric current [19]. 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 [12, 13]. The molecular basis of this phenomen is poorly understood.

The originality of this study consists of the combined therapies used for decreasing the PVY and PVX virus level (meristem culture, chemotherapy and treatments whit *S. hortensis* EOs and AO) and in the electrotherapy conditions used in our study (higher values for electric current).

So, this preliminary study revealed that chemotherapy (40 mg/l RBV + 40 mg/l OSMV), followed by treatments with *S. hortensis* EOs and AO of acclimatizated plants on the one hand and the electrotherapy (100mA, 10 minutes) on the another hand, had 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 'Roclas' cv.)
- the phytotoxicity of the treatments has to be verify
- to define the efficiency of the treatments with bulked samples is required

Further investigations are needed for combine chemotherapy + electrotherapy + treatments with EOs and AO of the acclimatizated plants, for improvement and optimization of these techniques, as well as understanding the mechanisms by which these therapies deactivates viral structures.

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