

EFFECT OF SOME THERAPIES ON POTATO PLANTLETS INFECTED WITH POTATO VIRUS X (PVX)

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Abstract: The purpose of this study is to decrease the 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) applied to microplants acclimatised in green house. The biological material used in experiments was plants (variety Roclas, virus free biological material) mechanically inoculated using: 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 l⁻¹ + OSMV 40mg l⁻¹; RBV 20mg l⁻¹ + OSMV 40 mg l⁻¹; RBV 20mg l⁻¹ + OSMV 80mg l⁻¹). The first variant (RBV40mg l⁻¹ + OSMV40mg l⁻¹ added to the tissue culture medium + essential oils treatments of acclimatised plants) and the electrotherapy variant 10minutes at 100mA showed the highest rate of virus eradication, the maximum values of the therapy efficiency.

Keywords: *Satureja hortensis* essential oils, potato virus X, Ribavirin, Oseltamivir, electrotherapy

1. Introduction

Potato virus X (PVX), a *Potexvirus*, 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.

Elimination of 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, electrotherapy) in eliminating PVX and producing virus-free plants (cultivar Roclas) was evaluated.

Untill now, many compounds were tested for their antiviral activity but few were effective (Schuster, 1988). The most used substance is the ribavirine (Virazol), an analogue of guanosine, wich when added to the medium at concentrations of 10-50mg/l, was effective against PVX, PVY, PVS and PVM in potato (Cassel and Long, 1982, Klein and Livingston, 1982; 1983; Cassel, 1987; Griffiths 1990). 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 (Klein and Livingston, 1982). Regeneration of potato sprouts from meristematic cultures was delayed by 6 to 8 weeks (Cassel, 1987). To overcome toxicity, a low dose of ribavirine was supplied with another compounds (antimetabolites). 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 ribavirine.

The treatments with *Satureja hortensis* essential oils and antioxidants (H_2O_2 and ascorbic acid) applied to acclimatised plants (obtained planting 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 (Bedoux et al., 2010). Maybe some compounds of these oils could be implicated in the processus signaling against stress, in infected potato plants (Bădărău, 2012; Bedoux, 2010).

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

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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 (Lozoya-Saldaña et al., 1996, Sabry et al., 2009, Hormozi-Nejad et al., 2010).

The study aimed to evaluate the therapy efficiency of different chemotherapies and treatments with *Satureja h.* EOs applied to acclimatised plants and several electrotherapies, for PVX elimination in potato plants and find out the best one for virus eradication.

2. 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 (Bădăraș et al., 2012) using a PVX secondary infected source cv. Ostara.

The plants had previously tested positive by ELISA for PVX, to confirm the occurrence of single infection 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 *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 containing a sterilized mixture of soil, vermiculite and organic matter (2:2:1). After 7 after the injection with EOs suspension (14 days for beginning the acclimatisation), the plants (excepting the controls) were sprayed twice a week with a *Satureja hortensis* essential oils suspension (1/1000, 5 ml each plant) (Bădăraș et al., 2012). The survivor plants were indexed after 45 days from the transfer in the green house.

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

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 l⁻¹ + OSMV 40mg l⁻¹ ; V2 = RBV 40mg l⁻¹ + OSMV 40 mg l⁻¹; V3 = RBV 20mg l⁻¹ + OSMV 80mg l⁻¹.

Table 1 Chemicals used for obtaining PVX virus-free plantlets

Chemicals	Activities	Chemicals Activities References
Ribavirin (Virazol) (1,β-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)
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)

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 sodium 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 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 aim to estimate an electrotherapy treatment leading to high rates of both virus elimination and plant regeneration, the Therapy Efficiency Index (TEI) was used (Lozoya-Saldaña et al. 1996). The TEI was estimated with the following relation:

$$TEI = \frac{\text{percentage of regenerated plantlets} \times \text{percentage of virus-free samples}}{100}$$

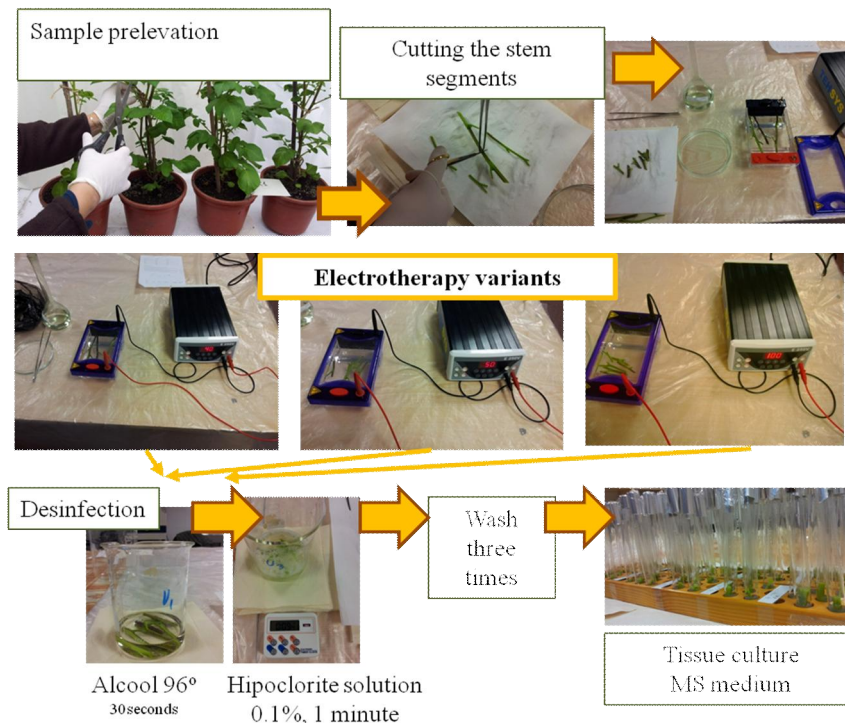


Fig.1. *Electrotherapy treatments steps and equipment used to produce virus-free plants in potato cv. Roclas: Power supply and electrophoresis tank used for producing electric currents, single node explants prepared for in vitro culture, electrotherapy variants (intensity of the electric current) regeneration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

3. Results and discussions

Chemotherapy. Results showed that in all the variants and stage of the therapy, for both viruses, plants virus free were found (table 2).

Regarding the PVX infected plants, the chemotherapy variant V2 (RBV 40mg/l + OSMV 40mg/l) combined with treatments with EOs and AO of acclimatised 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. 2). Highest

values for the virus elimination rate (100%) were obtained in variant V3 (RBV 20mg/l + OSMV 80mg/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. 2). The absorbances values at 405nm (DAS ELISA) for the plants acclimatized obtained from the variant V2 (RBV 40mg/l + OSMV 40mg/l) and treated wit EOs and AO were significantly lower (fig. 2).

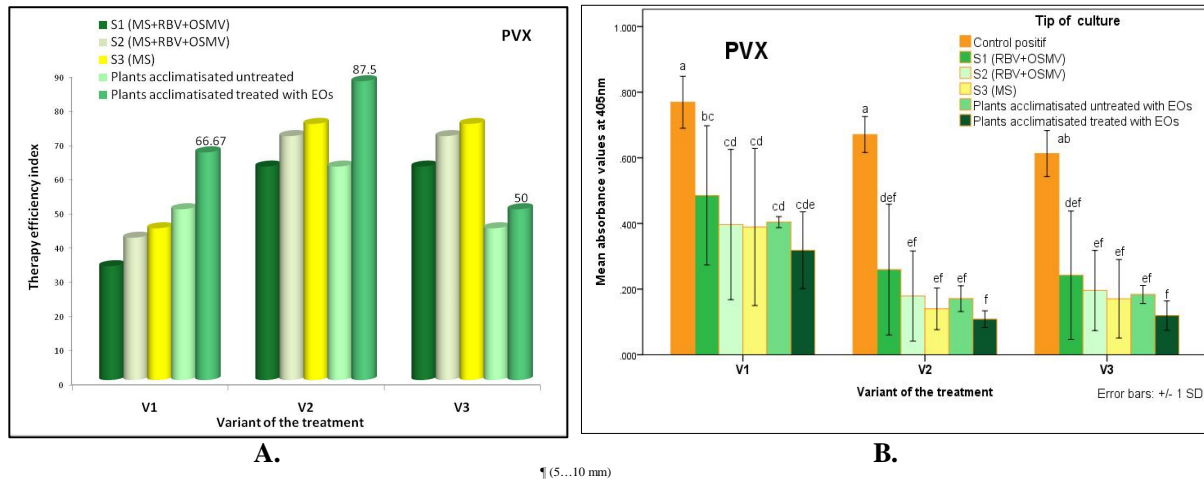


Fig. 2. Evaluation of chemotherapy and treatments with *Satureja hortensis* essential oils and H_2O_2 (1mM) on plants infected with potato virus X (PVX). The therapy efficiency of different variants and vegetation culture (B) The average values of absorbance in the plants regenerated from infected plants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

Table 2. Efficiency of chemotherapy, special treatments* of acclimatised 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	
		NPT/NRP	%	NPVF/NPM	%
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 acclimatised untreated	6/6	100	3/6	50
	Plants acclimatised 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 acclimatised untreated	7/8	87.5	5/7	71.4
	Plants acclimatised 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 acclimatised untreated	4/6	66.7	3/4	66.67
	Plants acclimatised treated *	3/6	50.0	3/3	100

V1= MS +RBV(20mg/L) + OSMV(40mg/L); V2 = MS +RBV(40mg/L) + OSMV(40mg/L); V3= MS +RBV(20mg/L) + OSMV(80mg/L); MS =Murashige and Skoog medium; RBV=Ribavirine; OSMV=Osetamivir; 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 H_2O_2 (1mM)

Electrotherapy

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). The figures 3,4 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. 4). But the success of electrotherapy in producing virus-free plants

depends upon both plant regeneration and virus elimination rates. 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 (Svetleva et al. 2003). The electric pulses are also reported as stimulants of plant differentiation *in vitro* (Hormozi-Nejad et al., 2010). It was demonstrated that regeneration of potato plant tissues could be improved by exposing explants to mild electric currents (Lozoya-Saldaña et al. 1996).

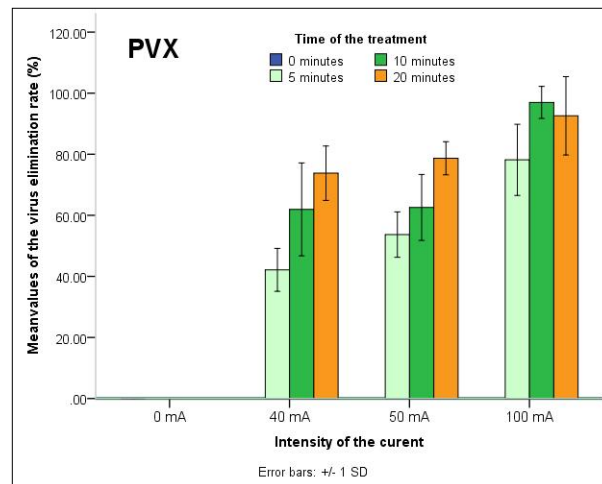


Fig. 3. Effects of electrotherapy treatments on the elimination rate of PVX in Roclas cv. infected material. Results = the mean of three experiments.

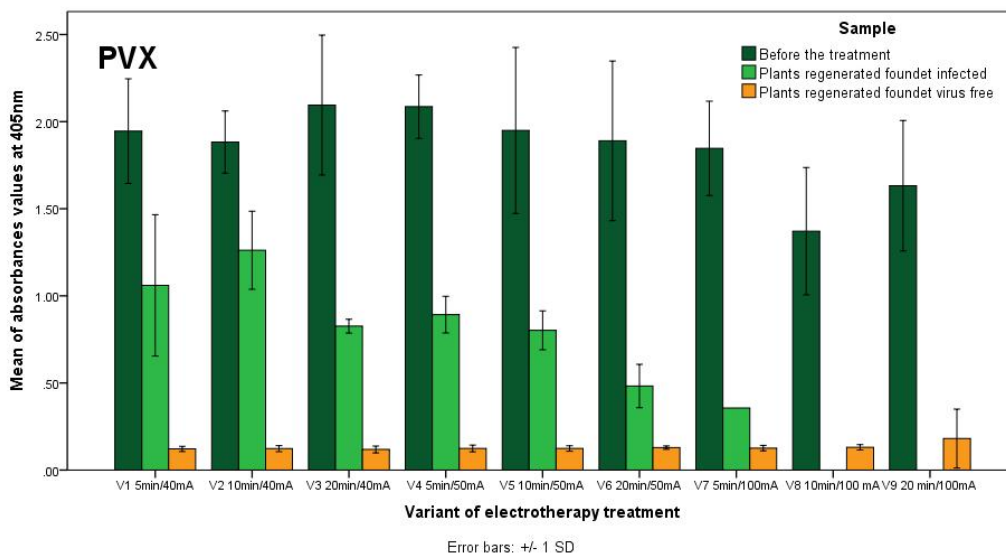


Fig. 4. Mean absorbances values of regenerated plants, using different electrotherapy treatments on potato plants (cultivars Roclas): OD in infected plants before electrotherapy (green dark bars) and in regenerated plants after electrotherapy which were ELISA positive (green bars) and ELISA negative (orange bars). Results = the mean of three experiments.

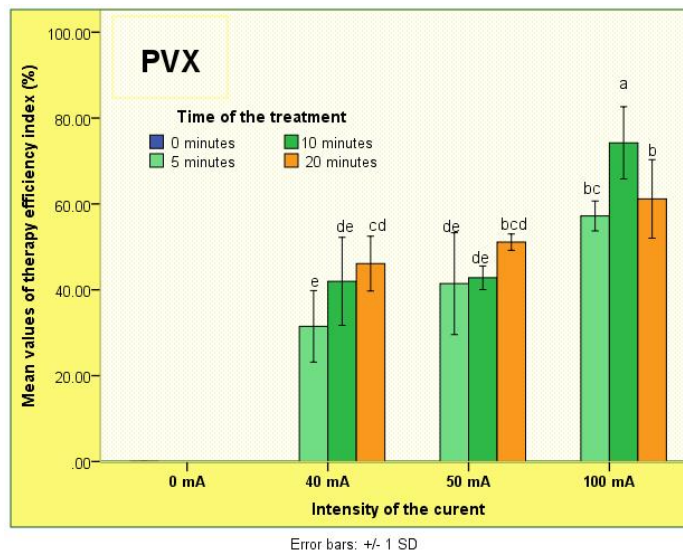


Fig. 5. Effects of electrotherapy treatments on the Roclas cultivar infected explants. Mean of the therapy efficiency index (TEI) for PVX infected and treated material. Results = the mean of three experiments. Bars with different letters differ significantly by ANOVA and Duncan's test ($P < 0.05$).

Plant regeneration rate estimated as the ratio of the number of regenerated plantlets to the total number of cultured explants was 56.1- 77.2%. Usually, higher intensities of electric current affected the survival of explants and thus plant regeneration.

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 (Jung-Yoon et al. 2003; Mahmoud et al. 2009). In our study we obtained good results regarding the regeneration rate when the oseltamivir was used or when the acclimatised plants were treated with *Satureja hortensis* EOs and AO.

Regenerated plantlets were tested for PVX infection by DAS-ELISA. A reduced concentration of virus was observed in all regenerated plantlets (fig. 4). On this basis, the mean virus elimination rates of Roclas cultivar explants exposed different periods to the three electric currents of 40, 50 and 100mA was 62/7%, 54.9% and 88.7% for PVX infected material (figure 3). 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 at 40, 50 and 100mA was estimated as 42.6, 44.6 and 64.3. The electric currents of 100 mA for 10 minutes resulted in the highest TEI value: 74.3 for PVX infected material (figure 5).

It has been postulated as a hypothesis that viral nucleoproteins may be denatured by when it is exposed to electric current (Lozoya-Saldaña et al. 1996). 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 (Hormozi-Nejad et al., 2010; Hull R., 2002). The basis of this phenomenon is still poorly understood.

4. Coclusions

This preliminary study revealed that chemotherapy (40mg/l RBV + 40mg/l OSMV), followed by treatments with *Satureja hortensis* EOs and AO of acclimatised plants on the one hand and the electrotherapy (100mA, 10 minutes) on the another hand, had effects on PVX elimination from potato plant tissues.

Further investigations are needed for combine chemotherapy + electrotherapy + treatments with EOs and AO of the

acclimatised plants, for improvement and optimization of these techniques.

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References

1. Bădăraș C. L., Mărculescu A., Chiru N., S. C. Chiru: *Benefic effects of essential oils treatments in healthy and potato virus Y infected plants of Solanum tuberosum L. (cv.Roclas) and Nicotiana tabacum(cv.White Burley)*. In: Romanian Agricultural Research, Vol. 29, 2012, p. 281-28
2. Bedoux G., Mainguy, C., Bodoux, M., F. Marculescu, A., Ionescu, D.: *Biological activities of the essential oils from selected aromatic plants*. In: Journal of EcoAgroTurism, Transilvania University of Brasov, Vol. 6 (1), 2010, p. 92-100
3. Cassel A. C. and Long R.D.: *The elimination of potato virus X, Y, S and M in meristem and explants cultures of potato in the presence of virazole*. In: Potato Research 25, 1982, p. 165-173
4. Cassel, A. C.: *In vitro induction of virus-free potatoes by chemotherapy*. In: Biotrechnology in Agriculture and Forestry, Vol. 3. Potato (ed.) Y.P.S. Bajaj, p. 40-50, Springer-Verlag, Berlin, Germany, 1987
5. Clark, M.F. and A.N. Adams: *Characterization of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant virus*. In: Journal of General Virology, 34, 1977, p. 475-483.
6. Griffiths, H.M., S.A. Slack and J.H. Dodds, : *Effect of chemical and heat therapy on virus concentrations in in vitro potato plantlets*. In: Canadian Journal Botanic, 68, 1990, p. 1515-1521
7. Hormozi-Nejad, M. H., Mozafari J. & Rakhshandehroo F.: *Elimination of Bean common mosaic virus using an electrotherapy technique*. Journal of Plant Diseases and Protection, ISSN 1861-3829, 117 (5), 2010, p. 201-205
8. Hull R.: *Induction of disease: Virus movement through the plant and effect on plant metabolism*. In: *Matthew's Plant Virology*, Fourth Edition, 2002, p.373-411
9. Jung-Yoon, Y., S. Hyo-won, C. Young-Mee, P. Young-Eun: *Ribavirin, electric current and shoot tip culture to eliminate several potato viruses*. In: Journal of Plant Biotechnology, 5, 2003, p. 101-105.
10. Klein, R. E. and Livingston, C. H.: *Eradication of potato virus X from potato with ribavirin treatment of cultured potato shoot tips*. In: American Potato Journal 59, 1982, p. 359-365
11. Klein, R.E. and Livingston, C.H.: *Eradication of potato viruses X and S from potato shoot-tip cultures with ribavirin*. In: Phytopathology 73, 1993, p.1049-1050
12. Lozoya-Saldaña, H., F.J. Abelló, G. García: *Electrotherapy and shoot tip culture eliminate potato virus X in potatoes*. In: American Potato Journal, 73, 1996, p.149-154.
13. Mahmoud, S.Y.M., M.H. Hosseiny, M.H. Abdel-Ghaffar: *Evaluation of some therapies to eliminate potato Y potyvirus from potato plants*. In: International Journal of Virology, 5, 2009, p. 64-76.
14. Murashige, T. and F. Skoog: *A revised medium for rapid growth and bioassays with tobacco tissue cultures*. In: Physiologia Plantarum, 15, 1962, p. 473-497
15. Sabry Y.M., Mahmoud, Maher H. Hosseiny and Mamdouh H. Abdel-Ghaffar: *Evaluation of some therapies to eliminate Potato Y Potyvirus from potato plant*. In: International Journal of Virology, 5, 2009, p.: 64-76
16. Schuster, G.: *Antiphytovirale Verbindungen mit Guanidinstruktur*. In: Phytopathologgy Z. 103, 1982, p.44-86
17. Schuster, G.: *Synthetic antiphytoviral substances*. In: Applied Virology Research 1, 1988, p. 265-283
18. Steward Gray, Solke De boer, Kames Lorenzen, Jonathan Withworth, Philipe Nolte, Rudra Singh, Alain Boucher, Huimin Xu. 2010. *Potato virus Y an evolving concern for potato crops in the United State and Canada*. In: Plant Disease / Vol. 94 No. 12, 2010, p. 1384-1397
19. Svetleva, D., M. Velcheva, G. Bhowmik: *Biotechnology as a useful tool in common bean (Phaseolus vulgaris L.) improvement*. In: Euphytica, 131, 2003, p. 189-200.
20. Ward, P., Small, I., Smith, J., Suter, P., Dutkowski, R.: *Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic* In: The Journal of antimicrobial chemotherapy 55 (Suppl 1), 2005, p. 5-21.

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SUMMARY

	Editorial	
	1	Academic Message
 		Biodiversity 9 <i>Scientific basis of a national, strategic and preference interest project, of agri-food bioeconomy and of tourism eco-economy, by innovative valorization of the Romanian territorial capital in the 21st century</i> A. T. Bogdan, R. Gruia, G. F. Tobă, C. Paşalău 11 <i>Effect of some Therapies on potato plantlets Infected with Potato Virus X (Pvx)</i> C. L. Bădărău, N. Chiru 18 <i>A Laboratory Method for the Detection of Mechanical Damages at potato sorting</i> F. V. Edu, D. M. Dănilă 22 <i>Results on the Behaviour of some potato varieties (Solanum Tuberosum L.) Suitable for Industrial Processing at the Potato Research and Development Station Targu Secuiesc</i> G. Mike 28 <i>Plant Sources Useful In Digestive Enzyme Preparations</i> A. Cozea, V.Tamas, M. Popescu, M. Neagu, N. Bordei
   		CalitaTerra 32 <i>Bulk Products Separation on a Stepped Screen Surface</i> O. Falko 37 <i>Studying of the Compressive Strength of Corrugated Paper Board by using Fem-Based Computer Simulations</i> S. Stefanov, V. Hadjiiski, D. Gospodinov 41 <i>Research regarding convective drying of fruits</i> N. Ţane, W. Thierheimer, Ghe. Duruiian 47 <i>Using Oo Granulometric Distribution Functions in estimating Flows of Grist sorted in a Plansifter Compartment</i> Gh. Voicu, G.A. Constantin, E.M. Ştefan, T. Căsândroi 53 <i>Investigation of the influence of the process and the medium properties conditions on the kinetics of green buckwheat flakes swelling process</i> L. Glagoleva, I. Korotkikh 56 <i>Research of Quality Indices of Drinking cow milk</i> V. Malygina, V. Sukmanov, Y. Petrova, E.V. Bulgakova, K. Krotynova, D. Afenchenko, D. Dimitrov, I. Kostova, S. Damyanova, N. Ivanova, M. Petrova 66 <i>Drying of plant materials in a Vibro-Fluidized Bed with Infrared Heating</i> A.N. Poperechnyi, I.V. Zhdanov, N.O. Mironova, A.V. Shulga 71 <i>Cavitation Treatment of Grape Rakia</i> D. Stoeva, M. Angelov 75 <i>Observation of the Aero-Dynamic Characteristics of Corrugated Wall Tube</i> D. Stoeva

Sanogeneous Food	
	<p>83 <i>Effect of some Technological Treatments of Cold Pressed Walnut Oil (<i>Juglans Regia</i> L.) on its Antioxidant Profile and Quality Characteristics</i> C. Popovici, P. Tatarov</p>
	<p>90 <i>Overview of National Moldavian Regulations on Food Labeling</i> C. Popovici, E. Dmitrieva, A. Birca, I. Iatco</p> <p>96 <i>Food labels – an opportunity for informed choice of food</i> M. Petrova, I. Kostova, N. Ivanova, I. Ivanova, S. Damyanova</p> <p>103 <i>Research of Process of Output of Foamed Mixtures Made of Raw Fish</i> A. Yashonkov, V. Sukmanov</p>
	<p>109 <i>Metal Levels in Wine between Toxicity and Beneficial Effects on Human Health</i> G. Vasile Scaeteanu, R.M.Madjar, C.Calin</p> <p>118 <i>Perspective on Human Exposure to Pesticides and their Metabolites in Different Media</i> R.M. Madjar, G.Vasile Scaeteanu, C.Calin</p>
	<p>129 <i>Do young people understand the information on Food Labeling?</i> Y. Stefanova, S. Stefanov, G. Antova</p> <p>133 <i>Research on Optimising the Production Technology of Ice Wine at the Târnave Vineyard, Jidvei Wine Centre</i> C. Tana, C. Marginean, O.Tița, M. Tița</p> <p>139 <i>The Phenolic Content of red wines from Târnave Vineyard, in 2010-2011-2012</i> C. Mărginean, C. Tana, O. Tița, A. Tița</p>
	<p>143 <i>Comprehensive Evaluation of Quality of milk and vegetable mince with buttermilk Concentrate</i> T. Yudina, I. Nazarenko</p> <p>150 <i>A Study in Beer Pasteurization with the Help of Microwaves</i> M. Tomescu (Cismărescu), C. Csatlós</p> <p>156 <i>Hplc Studies on Optimum Technological Conditions to obtain garlic powder, rich in Allicin, from garlic bulbs for Phytotherapeutic products</i> V. Luntraru, V. Tamaș, Șt. Manea, C. Luntraru</p>
Sustainable Tourism and Hospitality	
	<p>160 <i>Educational Experiences of a Tourism Softwer (Front Office System) at the University of Debrecen</i> G. Ráthonyi, L. Várallyai, I. Polyák Nagyné, K. Pető</p> <p>169 <i>Conception of an Integrated System for Agri-Tourism at Secusigiu (Arad County)</i> D. Mnerie, D. Țucu, M. Muntean, F. Sidea</p>
	<p>174 <i>The Rules of ICT in Regional Development Related to the Agri-Food and Tourism Sectors</i> M. Herdon, K. Pető, Sz. Botos, L. Várallyai</p> <p>182 <i>Econometric Study of the Evolution of Brasov Lodging Industry and Tourism Market</i> G. I. Florescu</p>

 	Ideas and Concepts 192 <i>Development of Biotechnology Combined Food Systems Enriched with Plant Complexes on the Basis of Animal Origin</i> N. Rodionova, M. Manukowskai, E. Popov, E. Shitakova, M. Popova 195 <i>On a new Relationship Between the Energy Consumption and Particle Size in a Processes of Crushing and Grinding</i> A.A. Ospanov 198 <i>Vacuum-Sublimation Drying of National Milk Drinks – Shubat And Koumiss – when Imposing Magnetic and Super High-Frequency Fields</i> T. A. Kulakhmetovna 201 <i>Study on the Compliance with Eu Food Labeling Regulations in Romania</i> O. Tița, M. Ognean, R.M. Iancu, C.F. Ognean, M.A. Tița, C. Georgescu* 211 <i>Integronic Food Concept</i> R. Gruia
	News 219 <i>Department of Eco-biotechnologies ana Equipment for Food and Agriculture</i>



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