

THE CHLOROPHYLL AND ANTHOCYANIN CONTENT OF REGENERATED POTATO PLANTS OBTAINED FROM PVX AND PVY INFECTED PLANTLETS TREATED BY COMBINED THERAPIES (PRELIMINARY STUDIES)

Carmen Liliana BĂDĂRĂU^{*,**}, Andreea NISTOR^{**}, Florentina STROE^{**}

^{*} Faculty of Food and Tourism, Transilvania University, Braşov, Romania

^{**} National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania

Corresponding author: Carmen Liliana Badarau, Faculty of Food and Tourism, Transilvania University, Brasov, 2 Castelului, 500470 Brasov, Romania, tel. 0040268476795, fax: 0040268476608, e-mail: carmen.badarau@unitbv.ro

Abstract. The purpose of this study was to estimate the effects of combined therapies (electrotherapy and treatments with *Satureja hortensis* essential oils, H₂O₂ 1mM pH 5.6 applied by spraying the plants acclimated in green house for decrease PVX and PVY infection level. Electrotherapy was applied in 4 variants: after washing and sizing explants, potato stems infected were exposed to 100 and 50 miliampers, for either 10 or 20 minutes, followed by sterilization and planting the axillary buds tip *in vitro*. Only the healthy plants (obtained from PVY and PVX infected plantlets variety Roclas) were used for the experiment. Monitoring the vegetative state of regenerated plant was done by estimation the chlorophyll (portable device SPAD 502 Chlorophyll Meter) and the anthocyanin (portable device ACM 200 plus, Antocianin Chlorophyll Meter) content of the leaf. Within the decrease of PVY and PVX infection level by using electrotherapy and was noticed a significant decrease of chlorophyll content compared to the negative control in case of variant 100mA/20minutes. Regarding the content of anthocyanin, there were not significant differences between values recorded in the experimental variants. Compared to the negative control, however, it was found small increase of anthocyanin content in case of material initially infected with PVY (but the values were not statistically supported). As opposed the content of anthocyanin, we noticed that monitoring of chlorophyll content indicated beneficial effects of combined therapies over the biological material regenerated from plantlets infected with PVX and PVY and treated.

Keywords: PVX, PVY, electrotherapy, chlorophyll, anthocyanin, *Satureja hortensis* essential oils

Abbreviations: PVY potato virus Y; PVX potato virus X; EOs essential oils; MS Murashige Skoog

INTRODUCTION

Potato virus Y (PVY), genus Potyvirus, member of the *Potyviridae* family, is an important pathogen for a wide range of plant species, primarily from the family *Solanaceae*. PVY in potato has received a lot of attention because this virus is the most economically important disease problem in seed potatoes in many areas of the world [21, 22, 26]. The virus is responsible for decreases in yield and quality, but the main issue in seed potato production is a requirement for strict virus tolerance limits for certified seed. High levels of PVY have been responsible for many seed lots being rejected as certified seed, resulting in a significant reduction in crop value, and at times in a shortage of certified seed, especially of certain cultivars that are highly susceptible to infection [1, 4, 8, 23].

Potato virus X (PVX), genus *Potexvirus*, family *Alphaflexiviridae* 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. Within the proceedings concerning the phytosanitary certification, its incidence in the post-harvest tests is multiplied by the coefficient 0.33 [24]. When *potato virus Y* is present, synergy between these two viruses causes severe symptoms in potatoes [2, 3]. Elimination of PVX from potato crop is essential for seed potato production. Also, in several studies, the efficiency of some techniques (chemotherapy, electrotherapy) in eliminating PVX and producing virus-free plants (cultivar Roclas) was evaluated [2 - 4]. But it is very important to know the effects of these treatments on the plants development [2 - 4].

The treatments with *Satureja hortensis* essential oils, H₂O₂ and ascorbic acid applied to acclimated plants could be beneficial for obtaining virus free material [4]. The essential oils from *Satureja hortensis* L. are known for its antiseptic (antifungal and antiviral) properties [5]. Maybe, some compounds of these oils could be implicated in the responses against stress, in infected potato plants [1, 4, 5]. Plant cells have defensive responses to pathogen attack associated with changes in oxidative metabolism [12]. H₂O₂ is believed to play two distinct roles in pathogenesis: one involves the oxidative burst in the hypersensitive response, which restricts pathogen growth [16, 20] and the other activates plant 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) [20].

The methods employed to eliminate viruses from plants like meristem culture, chemotherapy and thermotherapy are technically demanding and time consuming. Although the corona electric discharge is known and intensively studied for its technological applications (electrophotography, electro precipitation) works on the effect of corona discharge on living matter are relatively few and recent [10, 11].

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 [13, 17, 21]. Maybe, several nucleoprotein from plant cells could be affected, the treatments leading to accidental, undesired genetic modifications in the development of plantlets (*in vitro*), but we didn't

find until now, in the literature, references regarding this observation. 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 [2-4, 13, 15, 17, 18].

It has been postulated as a hypothesis that viral nucleoproteins may be denatured by when it is exposed to electric current [17]. 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 [13, 14]. The basis of this observation is still poorly understood.

The study aimed to evaluate the effects (on chlorophyll and anthocyanin content) of several therapies (treatments with *Satureja hortensis* oils, H₂O₂ and ascorbic acid, electrotherapy) used for PVX and PVY elimination in potato plants and find out the best one both for an optimal next plants development. Another objective of this preliminary study was to verify if these combined therapies (especially the treatments with oils suspensions, H₂O₂ and ascorbic acid) had effects on the content of photosynthetic pigments, which may affect also the plant development and therefore the values of some specific yield parameters (us presented by other researchers [6, 25]).

MATERIALS AND METHOD

Plants material. *Solanum tuberosum* L. plantlets 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 [19] at 20±1°C under a 16 h photoperiod (fluorescent lights, 400–700 nm). The plantlets 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, 2, 4] using a PVY secondary infected source cv. Record (PVY^o) and respectively a PVX secondary infected source cv. Ostara [4].

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 (sub-culture S1-22 days, sub-culture S2 -28 days). The plantlets were planted in pots, under greenhouse conditions.

Acclimatization, treatments with *Satureja hortensis* essential oils and antioxidants [1, 4]

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) and kept under a transparent cover. After 7 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) and weekly with H₂O₂ (1 mM pH 5.6) and AA (3 mM pH 5.6) [2-4]. The controls were sprayed with distilled water. The survivor plants were indexed after 45 days.

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 sodium chloride solution (1M) in an electrophoresis tank and exposed to electric current using a power supply (Tehsys E250V) [2, 4]. The variants of electrotherapy (electric current intensity, time) used in this experiment are presented in table 1. After the treatment, the stems were surface sterilized 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 Murashige (MS) medium [19]. The experiment was repeated three times for each electrotherapy treatment. Stem segments excised from infected potato plants were transferred two times in MS medium (sub-culture S1-28 days, sub-culture S2 -24 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.

Table 1. Experimental variants (electrotherapy)

Number variant*	V5	V6	V8	V9
Treatment (mA/min)	50/10	50/20	100/10	100/20

* The number is similar with that used for the experiments presented in the last research papers [3, 4]

Evaluation of the chlorophyll and anthocyanin content using specific contact sensors was done by:

-evaluation the chlorophyll content of leaf (portable device SPAD 502 Chlorophyll Meter) [27]. The values indicated the **relative** content of the chlorophyll quantity present in the plants leaves, measured by the transmittance of leaf at the two wavelengths, 650 nm (red) and 940 (near infrared –NIR) (Fig. 1A).

-estimation the anthocyanin content at leaf (portable device ACM 200 plus, Antocianin Content Meter) [28]. As in the case of determining the chlorophyll content, values indicated by device represents the **relative** sum of anthocyanin quantity present in leaves, estimated by transmittance of plant material, measured at two wavelengths, characteristic for anthocyanin pigment analysis (510nm and 700nm) (Fig. 1B).

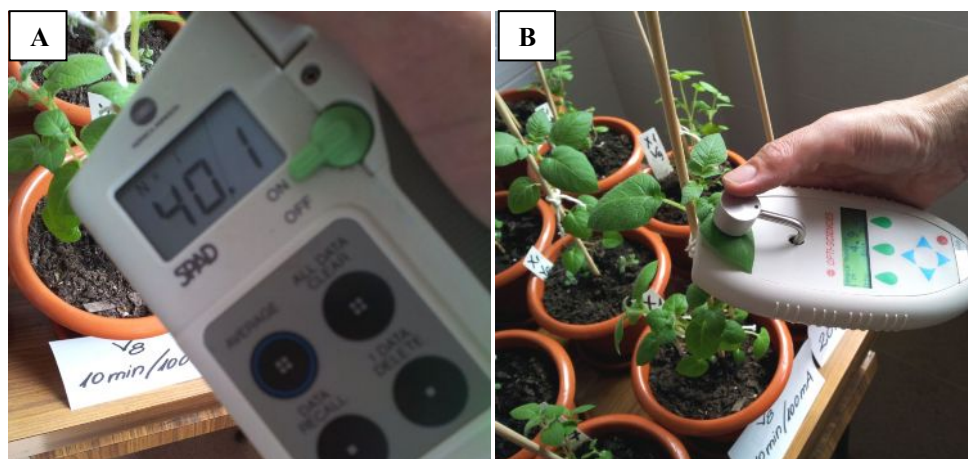


Figure 1. Estimation the chlorophyll content of leaf portable device (SPAD 502 Chlorophyll Meter) (A) and the anthocyanin content at leaf (portable device ACM 200 plus, Antocianin Chlorophyll Meter) (B) to plants obtained from plantlets treated by electrotherapy (variant V8).

Also, the values (determined using specific contact sensors) indicated the relative content of the the photosynthetic pigments content in the potato plants leaves.

The ACM-200-plus Anthocyanin Content Meter provides a fast determination of anthocyanin content on the intact leaves of plants, reducing also grinding or destructive assays. The measurement is fast, non destructive and simple to made. Laboratory methods for determination of anthocyanin content are both time consuming and destructive to the sample. Typically, a sample must be detached, ground up in a solvent and after that assayed using a spectrophotometer.

Anthocyanin has distinct optical absorbance characteristics that the ACM-200-plus exploits in order to determine relative anthocyanin concentration. The ACM-200-plus uses transmittance to estimate the anthocyanin content in leaf tissue according to the formula:

$$ACI = \frac{Transmit(931nm)}{Transmit(525nm)} \quad (1)$$

One wavelength falls within the anthocyanin absorbance range, while the infrared band serves to compensate sample thickness. The instrument measures the transmittance of both wavelengths and calculates an ACI (anthocyanin content index) value [28]. The tests were made after 14 and 28 vegetation days from the first treatment with EOs.

DAS ELISA test. The resulting plants were tested for the viruses using DAS-ELISA kits for PVY and PVX and according to the manufacturer’s instructions with several exceptions (Bioreba, Swiss). Also, the analysis was performed following the protocol described by [7] (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 (Magellan software). The samples having A_{405} values exceeding two times the average of healthy controls were considered virus infected.

Statistical analysis. Data were analysed by ANOVA and Duncan’s Multiple Range Test and scored as significant if $P < 0.05$ (IBM SPSS Statistics software).

RESULTS

The simple correlation coefficient Pearson revealed significantly higher values regarding chlorophyll content (as compared to the negative control) in the case of plants regenerated from the infected material with PVX, variant 100mA/10minutes. Plants regenerated from material infected with PVY virus behaved differently, the chlorophyll content being for variant 100mA/10minutes similar with that for the negative controls (Table 2).

Table 2. The correlation between content of chlorophyll pigment and anthocyanin at the leaf and kind of virus, inoculated on original material or variant of treatment

Variables (pigment tested)	Statistical indicators	Material used for obtaining regenerated plants (Healthy, PVX infected, PVY infected)	Variant of treatment (Healthy, 100mA/5min, 100mA/10min, 100mA/20min)
Chlorophyll content (units ACI)	Correlation coefficient Pearson	-0.374**	-0.245
	Significance threshold	0.005	0.074
	N	38	38
Anthocyanin content (units AAI)	Correlation coefficient Pearson	0.226	0.138
	Significance threshold	0.100	0.319
	N	38	38

For each variant were tested 5 regenerated plants, for each plant was determined mean value for three determinations (in different parts of foliage). ** Correlation is significant for $p < 0.01$.

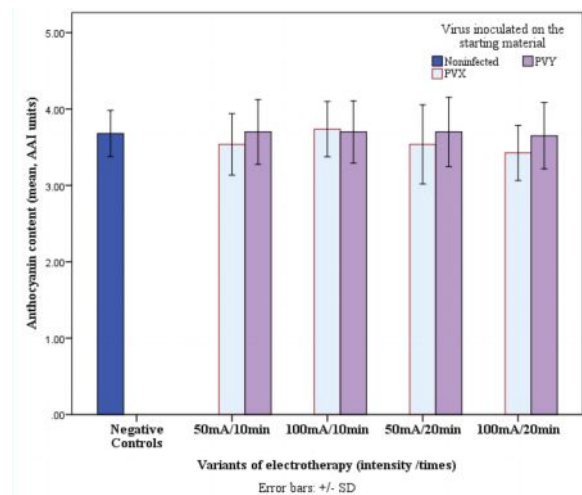


Figure 2. Electrotherapy treatments influence on anthocyanin content (specific contact sensors) of plants coming from biological material (from Roclas variety) infected with viruses PVY and PVX and regenerated after 2 consecutive subcultures (treated by electrotherapy before the first subculture, inoculated on MS medium) acclimated under greenhouse conditions and treated with EOs and AO.

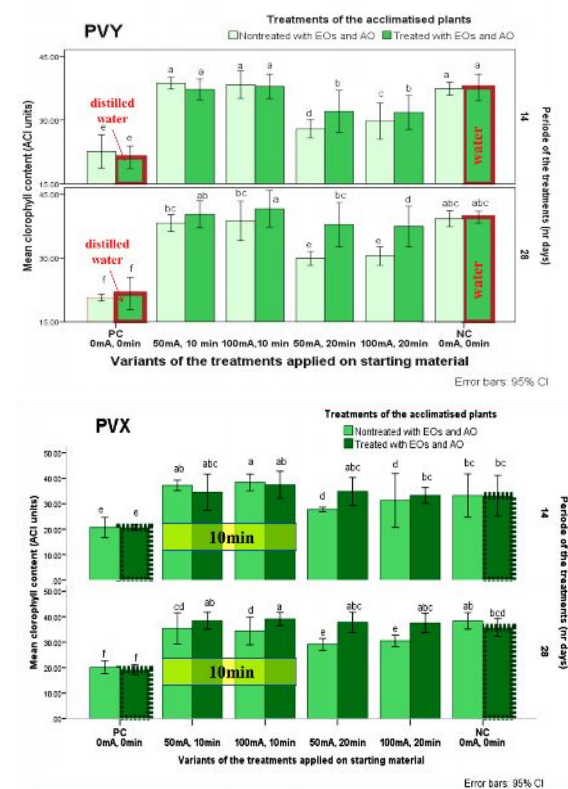


Figure 3. Effects of combined therapies (electrotherapy, treatments with essential oils, H₂O₂ and ascorbic acid) on chlorophyll content of plants obtained from infected material (Roclas variety) regenerated after 2 consecutive subcultures (electrotherapy before the first subculture, MS medium) and acclimated under greenhouse conditions. Chlorophyll content for plants obtained from PVY infected material (A) and from PVX infected material (B). The letters indicate significance of differences between variants (in each column for each type of virus) according to ANOVA and Duncan test (P < 0.05).

Within the elimination of viruses PVY and PVX by electrotherapy (data presented in the other papers [3, 4]) there was noticed a significant decrease of chlorophyll content compared to the control in case of variant 100mA/20minutes. The evolution of values for the mean number of leaves and the mean length of the plant (results presented recently [3]) was similar to chlorophyll content estimated for regenerated plants. Regarding the content of anthocyanin, there were no significant differences between values recorded in the experimental variants as was checked using simple correlation coefficient Pearson (Table 2).

Compared to the negative control, however, it was found small increase of the relative anthocyanin content in case of material initially infected with PVY (but the values were not statistically supported) (Fig. 2).

As opposed the content of anthocyanin, the results remark significantly differences between the variants regarding the relative chlorophyll content of, being observed effects of electrotherapy treatments over plantlets regenerated from material inoculated with viruses PVX and PVY.

DISCUSSIONS

The data obtained in this study could be useful for establish the correlations of the relative content of the photosynthetic pigments with different parameters of regenerated plants growth and with some yield specific indicators. Such correlations have been studied in other papers [6, 25]. Considering lowering of viral infection in case of treatment carried out in this experiment (already data presented [1-4]), the study intended to verify if the combined treatments applied affected the content of photosynthetic pigments, these having repercussions on subsequent parameters of plants growth [6, 24] and then on the specific yield indicators (mini tubers number and weight). At the same time, this paper tried to present a simple method for estimating the relative content of photosynthetic pigments, non-invasive methods, although the values shown are relative.

Between experimental variants were observed differences concerning plant development acclimated. Probably that, the severity of electroshocks treatment initially applied to biological material contributed to the differential development of acclimated plant. The first observations revealed pronounced effect that had particularly current intensity, respectively exposure duration of plantlets to electrotherapy treatments.

Application of electrotherapy on the potato cultivar Roclas resulted in partial elimination of PVX and PVY from potato tissues when the most severe treatments were applied (100 mA for 10-20 minutes) [2-4]. The figure 3 showed that this biochemical indicators analysed were not very different in responding to electrotherapy applying to PVX or PVY infected material (excepting the variant 100mA, 10minutes). In spite of developing of many virus-free plants [2] increasing levels of biological development indicator

values were observed in all variants even if the regenerated plantlets remain infected [2-4]. The success of electrotherapy in producing virus-free plants depends upon both plant multiplication rates, upon the next development of the plants [3,4]. 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 [18]. The electric pulses are also reported as stimulants of plant differentiation *in vitro* [2, 8, 9]. It was demonstrated that regeneration of potato plant tissues could be improved by exposing explants to mild electric currents [15, 18].

The results of other research work [3, 4] 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 [9, 15, 18]. For PVY and PVX infected material, the studies presented in other papers [3, 4] noticed good results regarding the multiplication rate when higher intensities of the electric current was used (100mA/10minutes). In this situation, compared to the negative control, it was found small increase of anthocyanin content in case of material initially infected with PVY (but the values were not statistically supported). Evaluation of t indicate The electrotherapy treatments had effects on the chlorophyll relative content of plants regenerated from material infected with viruses PVX and PVY. But, the combined therapies (usefull for decrease the level of PVY and PVX infection level - as noticed the results of recently research work [3, 4]) proved the possibility to use these techniques for regenerate virus free plants.

As presented in the last papers [4], the greatest value for therapy efficiency index (TEI) was noticed in variant with plants regenerated from infected material exposed to the electric current 100mA for 10 minutes, transferred in tissue culture, acclimated and treated with Eos and AO [4]. In case of variant with plants regenerated from infected material exposed to the electric current 100mA for 20 minutes, despite the elimination of viruses PVX and PVY by electrotherapy [3, 4] the results of this research work noticed a decrease of chlorophyll content compared to the negative control.

The chlorophyll relative content of the leaves in all the regenerated plants obtained from infected material treated by electrotherapy (100mA or 50mA for 10 minutes), had significantly higher values compared with the other experimental variants. So, these preliminary results suggest that the electrotherapy treatments had beneficial effects over the plants regenerated from PVX and PVY material infected and treated in these 2 variants. At the same time, in this case, the content of photosynthetic pigments, (which could affect plant development and therefore the values of several specific yield parameters [6, 25]) were not

significantly affected by the treatments with *Satureja hortensis* essential oils, H₂O₂ and ascorbic acid.

Acknowledgements This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDS-UEFISCDI, project number 104/2012.

REFERENCES

- [1] Bădărău, C.L., Marculescu, A., Chiru, N., Damsa, F., Nistor, A., (2010): Studies regarding the effects of *Rosmarinus officinalis* oil treatments in healthy and potato virus Y (PVY) infected plants *Solanum tuberosum* L. Analele Universitatii din Oradea, Fascicula Biologie, 17(2): 253-257.
- [2] Bădărău, C.L., Chiru, N., (2014): Effect of some therapies on potato plantlets infected with potato virus X (PVX). Journal of EcoAgriTourism, 10(1): 11-17.
- [3] Bădărău, C.L., Damsa, F., Chiru, N., (2014): Effects of some electrotherapy treatments of PVX infected potato plantlets cv. Roclas, on several biological development indicators. Journal of Horticulture, Forestry and Biotechnology, 18(3): 25- 29.
- [4] Bădărău, C.L., Chiru, N., Guță, I.C., (2014): The effect of some therapies on potato virus Y and X infected *Solanum tuberosum* L. plantlets (cv. Roclas). Analele Universitatii din Oradea, Fascicula Biologie, 21(1): 24-32.
- [5] Bedoux, G., Mainguy, C., Bodoux, M.F., Marculescu, A., Ionescu, D., (2010): Biological activities of the essential oils from selected aromatic plants. Journal of EcoAgroTurism, Transilvania University of Brasov, 6(1): 92-100.
- [6] Botha, E.J., Zebarth, B.J., Leblon, B., (2006): Non-destructive estimation of potato leaf chlorophyll and protein contents from hyperspectral measurements using the PROSPECT radiative transfer model. Canadian Journal of Plant Science, 86(1): 279-291.
- [7] Clark, M.F., Adams, A.N., (1977): Characterization of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant virus. Journal of General Virology, 34: 475-483.
- [8] Danci, O., Ziegler, A., Torrance, L., Gasemi, S.D., Danci, M., (2009): *Potyviridae* Family - short review. Journal of Horticulture, Forestry and Biotechnology, 13(1): 410-420.
- [9] Danci, M., Danci, O., Mike, L., Baciuc, A., Olaru, D., Petolescu, C., Berbentea, F., David, I. (2012): Production of virus free potato plantlets. Journal of Horticulture, Forestry and Biotechnology, 16(1): 232-238.
- [10] Giosanu, D., Bărbuceanu, M., Iorga Simăn, I., (2005): Biofizica-fundamente și aplicații, Ed. Universitatii Pitesti, pp. 230-243.
- [11] Gonzales, J.E., Sanchez, R., Sanchez, A., (2006): Biophysical analysis of electric current mediated nucleoprotein interaction process. Centro Agricola, 2: 42-47.
- [12] Hammerschmidt, R., (2005): Antioxidants and the regulation of defense. Physiological and Molecular Plant Pathology, 66: 211-212.
- [13] Hormozi-Nejad, M.H., Mozafar, J., Rakhshandehroo, F., (2010): Elimination of Bean common mosaic virus using an electrotherapy technique. Journal of Plant Diseases and Protection, 117(5): 201-205.
- [14] Hull, R., (2002): Induction of disease: Virus movement through the plant and effect on plant metabolism. In: Matthew's Plant Virology, Fourth Edition, pp. 373-411.
- [15] Jung-Yoon, Y., Hyo-won, S., Young-Mee, C., Young-Eun, P., (2003): Ribavirin, electric current and shoot tip

- culture to eliminate several potato viruses. *Journal of Plant Biotechnology*, 5: 101-105.
- [16] López-Delgado, H., Zavaleta-Mancera, H.A., Mora-Herrera, M., Vázquez-Rivera, E., Flores-Gutiérrez, F.X., Scott, I.M., (2005): Hydrogen peroxide increases potato tuber and stem starch content, stem diameter and stem lignin content. *American Journal of Potato Research*, 82: 279-285.
- [17] Lozoya-Saldaña, H., Abelló, F.J., García, G., (1996): Electrotherapy and shoot tip culture eliminate potato virus X in potatoes. *American Potato Journal*, 73: 149-154.
- [18] Mahmoud, S.Y.M., Hosseney, M.H., Abdel-Ghaffar, M.H., (2009): Evaluation of some therapies to eliminate potato Y potyvirus from potato plants. *International Journal of Virology*, 5: 64-76.
- [19] Murashige, T., Skoog, F., (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. In: *Physiologia Plantarum*, 15: 473-497.
- [20] Quan, L.J., Zhang, B., Shi, W.W., Li, H.Y., (2008): Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *Journal of Integrative Plant Biology*, 50: 2-18.
- [21] Quenoille, J., Vassilakos, N., Moury, B., (2013): Potato virus Y: a major crop pathogen that has provided major insights into the evolution of viral pathogenicity. *Molecular Plant Pathology*, 14: 439-452.
- [22] Scholthof, K.B., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, B., Saunders, K., Candresse, T., Ahlquist, P., Hemenway, C., Foster, G.D., (2011): Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology*, 12: 938-954.
- [23] Steward, G., De Boer, S., Lorenzen, K., Withworth, J., Nolte, P., Singh, R., Boucher, A., Xu, H., (2010): Potato virus Y an evolving concern for potato crops in the United State and Canada. *Plant Disease*, 94(12): 1384-1397.
- [24] Rasocha, V., Hausvater, E., Dolezal, P., (2008): Harmful agents of Potato. *Abionoses, Diseases, Pests. Potato Research Institute Havlickuv Brod Ltd (ed.)*, pp. 66-67.
- [25] Viga, R., Huzsvaib, L., Dobosa, A., Nagy, J., (2012): Systematic measurement methods for the determination of the SPAD values of maize (*Zea mays* L.) canopy and potato (*Solanum tuberosum* L.). *Communications in Soil Science and Plant Analysis*, 43 (12): 1648-1693.
- [26] Wrobel, S., Wlasik, A., (2013): Seed Potato production in Poland. *American Journal for Potato Research*, 3: 260-268.
- [27] *** SPAD 502 Chlorophyll Meter - User Guide.
- [28] *** ACM-200-PLUS Anthocyanin Meter - User Guide.

Received: 24 August 2015

Accepted: 5 October 2015

Published Online: 7 October 2015

Analele Universității din Oradea, Fascicula Biologie

<http://www.bioresearch.ro/revistaen.html>

Print-ISSN: 1224-5119

e-ISSN: 1844-7589

CD-ISSN: 1842-6433

University of Oradea Publishing House