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Application of Electrotherapy for the Elimination of Potato Potyviruses

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ABSTRACT

The use of healthy planting material is an important prerequisite for controlling destructive viral diseases in potato production. Virus elimination methods such as meristem culture and thermotherapy are usually used to produce the nucleus stock of healthy planting material. Here, we report a new technique of electrotherapy for elimination of two potyviruses, *Potato virus A* (PVA), and *Potato virus Y* (PVY), from potato plants. Electric currents of 15, 25 and 35 mili Ampers for 10 to 20 minutes were used for eliminating the combination of PVA and PVY in six potato cultivars. An electric current of 35 mili Ampers for 20 minutes was the most effective electrotherapy treatment for eliminating these two viruses. Responses of potato genotypes to electrotherapy were significantly different. Regenerations of electrotherapy treated plantlets in cultivars Lady Roseta and Banaba were 70.8 and 66.6 percent respectively, while these were approximately 54 to 58 percent for cultivars Olimpia, Agria, Desirea and Clone 69. Virus free plantlets were successfully produced in the potato cultivar Lady Roseta. However, in other cultivars examined in this study, electrotherapy resulted in drastic reduction of virus concentration but not total eradication of the virus infection from plant tissues.

Keywords: ELISA, Plant virus, Seed production, *Solanum tuberosum*, Tissue culture

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important dicotyledon plant in human nutrition. Among crop plants, potato species are hosts to the largest number of viruses. At least 37 viruses naturally infect cultivated potatoes (Beemster and de Bokx, 1987; Salazar 1996; Jeffries 1998). Some of these viruses, notably *Potato leafroll virus* (PLRV), *Potato virus A* (PVA), *Potato virus Y* (PVY) and *Potato virus S* (PVS) occur in potato crops worldwide, while others are important only in some geographical areas (Brunt, 2001). Viral diseases are easily transmitted via infected tubers from one generation to the next and from one region to another, causing considerable yield and quality losses. Conventional control methods of viral diseases which are based on the control of their viral vectors are generally economically and environmentally costly. Therefore, cultivation of disease-free seed tubers is considered the most effective method of controlling potato viruses. The use of healthy and certified seed potatoes has recently been increased around the world.

Application of tissue culture techniques is now a widespread practice in potato seed production systems in many countries (Farrell *et al*. 1982; Klein and Livingston 1982; Kwiatkowski *et al*. 1985; Faccioli and Colombarini 1996). Therefore, development of an efficient virus eradication technique

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and establishment of an *in vitro* collection of virus-free potato germplasm is a crucial prerequisite for production and certification of disease-free seed tubers (Mozafari and Pazhouhandeh, 2000). The efficiency of conventional methods of virus eradication from plants including meristem culture and thermotherapy is low and they are not successful for all viral infections. In addition, these methods have certain limitations due to time length requirement and less efficiency in producing virus-free plants. Alternatively, new techniques of chemotherapy and electrotherapy have proven to be successful in eliminating potato viruses in recent years. Chemotherapy was employed to eliminate PVS, PVY and PVX (Conrad, 1991; Faccioli and Zoffali, 1998; Pazhouhandeh, 2001), while electrotherapy was reported to be successful in eliminating PVX (Lozoya *et al* 1996), PVY and PLRV (Pazhouhandeh, 2001). Efficiencies of 60 to 100 percent were reported for electrotherapy in the production of virus-free plantlets from potato plants with a single infection of PVX (Lozoya *et al* 1996). Effects of electrotherapy, chemotherapy (with ribavirin) and meristem culture were studied comparatively by Pazhouhandeh (2001) indicating that the efficiency of each technique is dependent upon the cultivar-virus system and the intensity of the virus eradication treatment. Among different electric currents used for eliminating PVY and PLRV and production of virus free plants in potato, the electric current of 15 mili Ampers (mA) for 10 minutes produced the highest degree of virus elimination for PLRV, PVY and PVS (26% to 100%) (Pazhouhandeh *et al* 2001). Here, we report the efficiency of electrotherapy treatments in eliminating a dual infection of two potyviruses, PVA and PVY, and production of virus-free germplasm in six potato cultivars. Effects of cultivars and different electric currents and time courses of electrotherapy on the regeneration of virus-free plants were studied.

MATERIALS AND METHODS

Plant Materials

Seed tubers of six cultivated potato (*S. tuberosum*) varieties including Banaba, Olimpya, Agria, Desirea, Lady Roseta and Clone 69 of the Iranian potato breeding program were obtained and planted under controlled conditions in the greenhouse. Two-week-old plants were mechanically inoculated on their primary leaves with a crude leaf homogenate of positive control plants infected with PVA and PVY. Three grams of infected leaf tissue for each plant was first ground in the extraction buffer (0.01 M sodium phosphate with 0.4% sodium sulphite, pH 7.5) to provide a 1:5 dilution of sap. The homogenate was manually spread on the dorsal surface of leaf by gloved-fingers. The plants were grown in the greenhouse at a constant temperature of 24 °C with additional supply of 54 μmol m^{-2} s^{-1} light for 10 h provided by Na-High-Pressure lamps. Infection of inoculated plants was confirmed using an enzyme-linked immunosorbent assay (ELISA) at the end of the 7th week.

Detection of Viruses

Double Antibody Sandwich-ELISA was performed following a protocol described by Clark and Adams (1977). Microtiter ELISA plate wells were coated with antiserum diluted in carbonate buffer (pH 9.6) according to the supplier's (International Potato Centre, CIP) specifications. IgG used in the present experiment was diluted 1:300 (v/v) in the carbonate buffer. Plates were incubated for 3 h at 37 °C, followed by washing and then loading with plant extracts before incubation at 4 °C, overnight. After washing off the crude plant extract, viruses were detected by their corresponding antibodies conjugated with alkaline phosphotase and diluted in conjugate buffer (PBS-TPO, pH 7.4) according to the

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supplier's specifications. Optical density (OD) at 405 nm was measured by ELISA Reader (Stat Fax 2100, AWARNESS Tech. Inc., USA) 45 min to 1 h after incubation with the substrate ρ-nitrophenyl phosphate (1 mg ml^{-1}, pH 9.8). Tissue samples from healthy and infected plants grown *in vitro* were used as negative and positive controls, respectively. Infection of regenerated plants was determined according to R=X +2SD where, X is average OD of negative controls and SD is standard deviation of this mean relative to its individual wells.

Electrotherapy

The most vigorous stems of each genotype were selected and used for electrotherapy. Stem segments with approximately 3-5 axillary buds were treated at three different levels of electric currents (15, 25 and 35 mA) and two time courses (10 and 20 min) (Table 2) in an electrophoresis tank. Electric currents were supplied by an electrophoresis power supply. Electrotherapy-treated axillary buds were removed from stem segment and were cultured on MS basic medium (Murashige and Skoog, 1962) with half-concentration of inorganic salts and without plant hormones, after sterilizing with sodium hypochloride (Pazhouhandeh, 2001). Sub-culturing was carried out after 10 days using MS basic medium with full-concentration of inorganic salts. Plantlets were conserved in a growth chamber with a temperature of 23 °C during the day and 18 °C during the night, with a day length of 16 h, under 60% relative humidity and 54 µmol m^{-2} s^{-1} light intensity provided by fluorescent lamps. Effects of cultivars and electrotherapy treatments on virus elimination and regeneration of plantlets were studied in an *in vitro* factorial experiment with a completely randomized design in four replicates as (6×6)×4. Efficiency of therapy was estimated according to the following formula: efficiency of therapy = virus elimination percent × percent of plantlet regeneration (Pazhohandeh, 2001).

RESULTS AND DISCUSSION

Effects of Electrotherapy Treatments on Eliminating PVA and PVY

Plants were regenerated successfully from all electrotherapy treated cultivars. Analysis of variance on the ELISA OD readings of regenerated plantlets (Table 1) showed that the effects of both electrotherapy treatments and potato genotypes on the elimination of PVA and PVY were significant ($p< 0.01$). The significant effects of electrotherapy × cultivar interaction revealed that different potato genotypes responded differently to electrotherapy treatment (Table 1). In other words, there was no single electrotherapy

Table 1. Analysis of variance for cultivars and electrotherapy treatments for the elimination of PVA and PVY.

Sources of variation	Degree of freedom	Mean square	F
PVA:			
Cultivar	5	0.127	166.45**
Treatment	5	0.037	48.63**
Cultivar & Treatment	25	0.003	3.63**
Error	36	0.001	
PVY:			
Cultivar	5	0.079	97.92**
Treatment	5	0.041	51.15**
Cultivar & Treatment	25	0.004	4.97**
Error	36	0.001	

** Significant at $p<0.01$ level.

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Table 2. Regeneration rates of virus-free plantlets and the efficiency of electrotherapy treatments for the elimination of PVA and PVY.

Treatment	Electric currents(mA)	Duration (min)	Replicates	Regeneration (%)	Virus-free plantlets (%)	Efficiency of therapy
NA [a]	0	0	24	91.6	0	0
E1	15	10	24	62.5	0	0
E2	25	10	24	75	0	0
E3	35	10	24	66.6	8.3	199.2
E4	15	20	24	58	0	0
E5	25	20	24	50	0	0
E6	35	20	24	45.8	12.5	572.5

[a] Electrotherapy not applied.

treatment suitable for all genotypes. The treatment of 35 mA for 20 min was generally the most effective among the treatments in reducing the concentration of both PVA and PVY (Table 2). This treatment had, particularly, the highest effect on virus elimination from cv Lady Roseta followed by Agria, Olympia, Desirea, Clone 69 and Banaba, respectively. Not only genotypes but also viruses infecting a genotype responded differently to electrotherapy treatment. The minimum OD for PVA was obtained in plantlets derived from electrotherapy treatments of 35 mA, 25 mA and 15 mA for 20 minutes, whereas for PVY this was obtained at 35 mA for 10 minutes (Table 2).

Regenerated control plantlets without electrotherapy treatment were tested positive for PVA and PVY indicating that diminishing virus titer was due to electrotherapy, not to the regeneration *in vitro* (Figure 1). Earlier attempts to eliminate PVY using an electric current of 7 mA resulted, merely, in decreased levels of virus titer in regenerated plants (Yoon *et al.* 2003). However, in our earlier studies an electric current of 15 mA for 10 min was able to eliminate an important potato virus, PLRV, with a higher efficiency compared to potyviruses (Pazhouhandeh, 2001). In addition to the level and duration of electrotherapy, and plant genotype the efficiency of electrotherapy may be affected by structural differences among these viruses. PLRV has an isometric icosahedral body while the potyviruses, have flexuous particles (Hull, 2002; Pazhohandeh, 2001). In addition to the considerable limitations of conventional virus eradication methods in eliminating different viruses, the efficiency of these methods are also relatively low when compared with electrotherapy. The efficiency of therapy was reported to be three or five times higher for electrotherapy in comparison with conventional methods of thermotherapy or meristem culture, respectively (Lozoya *et al.,* 1996; Pazhohandeh, 2001). The electrotherapy technique described here was successful in eliminating two important potato potyviruses and can be further developed to be used for other potato cultivar-virus infection systems.

Regeneration of virus-free plantlets

Electrotherapy treatments affected potato genotypes differently both in elimination of viruses and regeneration of plantlets (Table 3). Adverse effects of electrotherapy may hamper the growth of potato plants depending on the genotypes. Therefore, for the production of virus-free plants, in addition to virus elimination, efficiency of plantlet regeneration is also equally important (Lozoya *et al.* 1996; Pazhouhandeh, 2001). The regeneration rate of plantlets was generally negatively affected by the intensity of electrotherapy treatment in such a way that the highest regeneration was observed in treatment E1 (15 mA for 10 min), followed by E2 (25 mA

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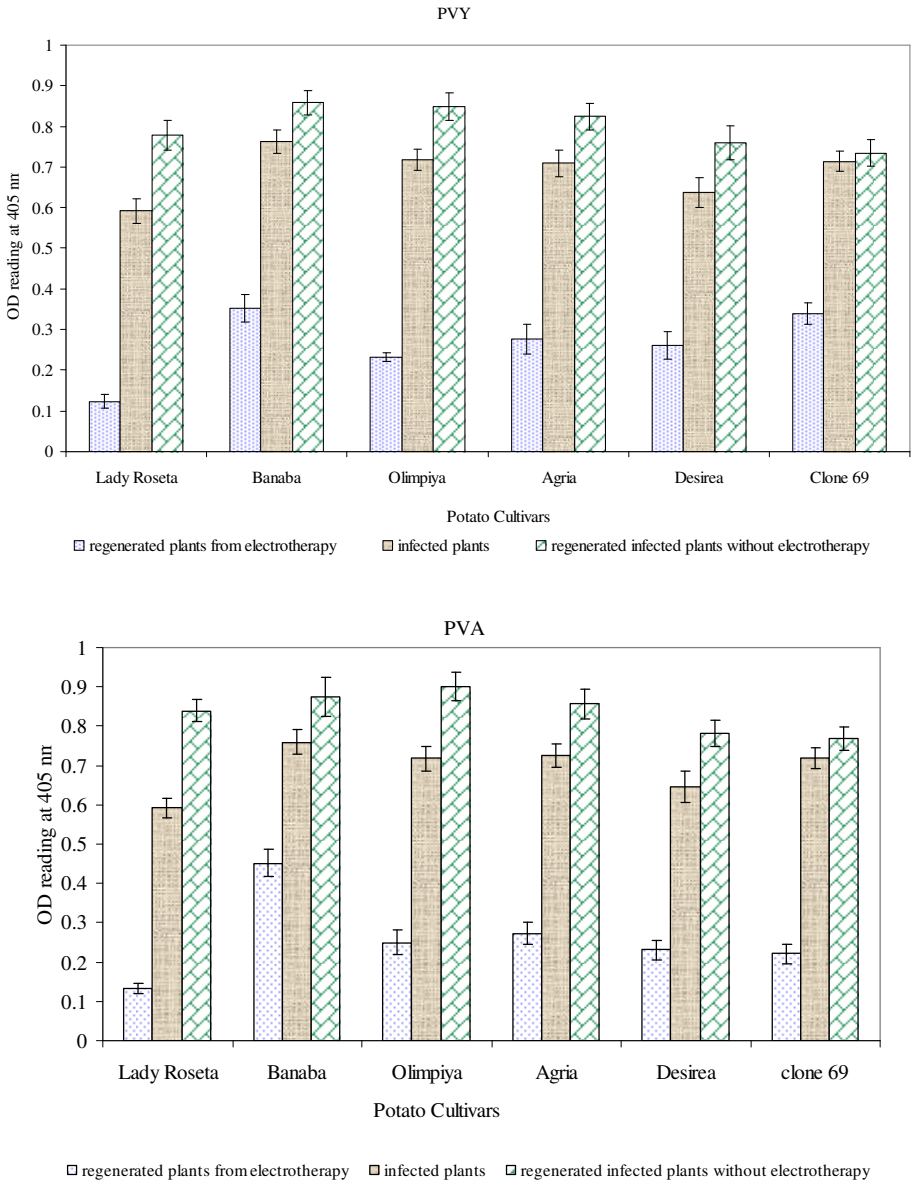

Figure 1. Mean ELISA OD readings for PVA and PVY in plantlets regenerated from different potato cultivars after electrotherapy.

for 10 mA) and E3 (35 mA for 10 min) (Table 2). Treatment E6 (35 mA current for 20 min) led to the lowest regeneration percentage of 45.8% while showing the highest virus elimination rate of 12.5% (Table 2). Due to the varying responses of potato genotypes to adverse effects of electrotherapy, the optimal treatment can be identified for each genotype-virus combination based on the rates of regeneration and virus elimination (Table 3). Virus-free plantlets were only regenerated from cv Lady Roseta for which a therapy efficiency of 1472.6 was estimated (Table 3). Percentage of regeneration varied between 45 and 75 among genotypes and electrotherapy treatments (Tables 2 and 3).

Similar levels of regeneration have also been reported in earlier studies, including regeneration rates of 40 to 80 percent (Lozoya *et al.* 1996) or 57 to 100 percent (Pazhohandeh, 2001) for potato cv Agria. No noticeable morphological differences were observed among regenerated plants (Mozafari

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Table 3. Regeneration rates of virus-free plantlets and efficiency of electrotherapy for eliminating potyviruses PVA and PVY from potato cultivars.

Cultivar	Replicates	Regeneration (%)	Virus-free plantlets (%)	Efficiency of therapy
Lady Roseta	24	70.8	20.8	1472.6
Banaba	24	66.6	0	0
Olimpya	24	54.1	0	0
Agria	24	58.3	0	0
Desirea	24	54.1	0	0
Clone 69	24	54.1	0	0

and Pazhohandeh, 2000). Only in one regenerated plant produced in this study abnormal growth was observed after electrotherapy. It seems that electrotherapy treatment actuates production of axillary buds in tissue culture plantlets. Some studies have speculated that electrical stimulation may induce physiological changes such as resistance to cold and drought and cause organogenesis in some crops. They have also shown that temperature can rise in the tissue from 4 to 10 ^{0}C, while pH of the cell remains unchanged before and after treatment (Lozoya *et al*. 1996).

Electrotherapy, compared to conventional virus elimination techniques of thermotherapy and meristem culture, is technically simple, faster and thus economically less costly. In addition, plant regeneration rate in electrotherapy is comparatively much higher (Pazhouhandeh *et al.* 2002). Based on our results, this method can be used as an effective technique for eliminating some viruses from some genotypes or species. However, conditions of electrotherapy may differ for each host-virus system. Therefore, it is essential to determine optimal electrotherapy treatment conditions for a desired host-virus system. Further studies are required to investigate the possible use of this technique in other plant species especially fruit trees and vegetatively propagated ornamental plants.

REFERENCES

1. Anonymous. 2003. Statistical analysis of potato production in 2002-2003. Office of Statistics and Information Technology, Ministry of Agriculture, Tehran, Iran.
2. Beemster, A. B. R., and De Bokx, J. A. 1987. Survey of Properties and Symptoms. In: "*Viruses of Potatoes and Seed Potato Production*" (Eds): de Bokx, J. A. and van der Want, JPH Pudoc, Wageningen, The Netherlands. pp. 84-113.
3. Brunt, A. 2001. Virus and Virus-like Diseases of Potatoes and Production of Seed-potatoes. In: "*The Main Viruses Infecting Potato Crops*" (Eds.): G. Loebenstein *et al.*, Kluwer Academic Publishers, The Netherlands. pp. 65-67.
4. Clark, M. F. and Adams, A. N. 1977. Characteristics of the Micro Plate Method of Enzyme –linked Immunosorbant Assay for the Detection of Plant Viruses. *J. Gen. Virol*. **34**: 475-483.
5. Conrad, P. L. 1991. Potato Virus S Free Plants Obtained Using Antiviral Compounds and Nodal Segment Culture of Potato. *AM Potato J*. **68**: 507-513.
6. Faccioli, G. and Colombarini, A. 1996. Correlation of Potato Virus S and Virus M Contents of Potato Meristem Tips with the Percentage of Virus-free Plantlets Produced in Vitro. *Potato Res.* **39**: 129-140.
7. Faccioli, G. and Zoffoli, R. 1998. Fast Eradication of Potato Virus X and Potato Virus S from Virus Infected Potato Stem Cutting by Chemotherapy. *Phytopath. Medit*. **37**: 9-12.
8. Farrell, C. J., Martin, M. W. and Thomas, P. E. 1982. Producing Virus-tested Free Potatoes by Thermotherapy and Meristem Culture. *Proc. Wash. State Potato Conf.* **21**: 57-62.
9. Hull, R. 2002. Matthews' Plant Virology. Academic Press. New York, pp. 33-36.
10. Jeffries, C. J. 1998. Potato. FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm No. 19. pp. 177.
11. Klein, R. E. and Livingston, C. H.1982. Eradication of Potato Virus X from Potato

[DOR: 20.1001.1.16807073.2011.13.6.15.8]

by Ribavirin Treatment of Cultivated Potato Shoots Tips. *AM Potato J*. **59**: 359-365.
12. Kwiatkowski, S., Martin, M. W. and Thomas, P. E. 1985. The Pacific Northwest Potato Clonal Repository. *AM Potato J*. **62**: 433.
13. Lozoya, H., Abello, F. and Garcia, G. 1996. Electrotherapy and Shoot Tip Culture Eliminate PVX in Potatoes. *AM Potato J*. **73**: 149-154.
14. Mozafari, J. and Pazhouhandeh, M. 2000. Biotechnology for Development of the First Iranian *in vitro* Gene-bank of Virus-free Potato Germplasm. Bulletin of Biotechnology Commission**, 3**: 166-173.
15. Murashige, T. and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bioassay with Tobacco Tissue Culture. *Physiol. Plant*. **15**: 473-479.
16. Pazhouhandeh, M. 2001. Establishment of in vitro Gene-bank for Virus-free Potato Germplasm. MSc Thesis, Tarbiat Modarres University.
17. Pazhohandeh, M., Mozafari, J. and Alizadeh, A. 2002. Electrotherapy a New Technique for Virus Eradication from Plants. In: Proceedings of the 15th Iranian Plant Protection Congress. Razi University of Kermanshah, Iran, pp: 189-190.
18. Salazar, L. F. 1996. Potato Viruses and Their Control. CIP, Lima, Peru, 214 pp.
19. Yoon, J., Wonseo, H., Mee Choi, Y. and Eun Park, Y. 2003. Ribavirin, Electric Current, and Shoot-tip Culture to Eliminate Several Potato Viruses. *J. Plant Biotechnol*. **5(2)**:101-105.

استفاده از الکتروتراپی برای حذف پوتی ویروسهای سیب زمینی

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چکیده

در گیاهان دارای تکثیر رویشی مانند سیب زمینی، استفاده از مواد گیاهی سالم یکی از مهمترین پیش نیازها جهت کنترل اثرات مخرب بیماریهای ویروسی بوده که به آسانی از یک نسل به نسل دیگر انتقال پیدا می کنند. در حال حاضر از روشهای معمول حذف ویروس مانند کشت مریستم و ترموتراپی برای تولید هسته های اولیه بذر و نهال سالم استفاده می شود. در این تحقیق روش جدید الکتروتراپی جهت حذف دو پوتی ویروس سیب زمینی PVA و PVY به کار برده شدند. جریانهای الکتریسیته ۱۵، ۲۵ و ۳۵ میلی آمپر در زمانهای ۱۰ و ۲۰ دقیقه جهت حذف آلودگی های مرکب PVA و PVY در شش رقم سیب زمینی مورد استفاده قرار گرفتند. جریان الکتریسیته ۳۵ میلی آمپر به مدت ۲۰ دقیقه موثرترین تیمار الکتروتراپی جهت حذف آلودگی مرکب دو ویروس بود. پاسخ ژنوتیپها به تیمارهای الکتروتراپی معنی دار بود. باززایی گیاهان الکتروتراپی شده در ارقام لیدی روزتا و بانابا به ترتیب ۷۰/۸ و ۶۶/۶ بود، درحالیکه این نسبت در ارقام المپیا، آگریا، دزیره و کلون ۶۹، ۵۴ تا ۵۸ درصد بود. در رقم لیدی روزتا گیاهچه های عاری از ویروس با موفقیت تولید شد، اگرچه در سایر ارقام مورد بررسی در این تحقیق، غلظت ویروس به شدت کاهش پیدا کرد اما منجر به حذف کامل ویروس از بافتهای گیاهی نشد.

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