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Evaluation of some therapies to eliminate potato Y potyvirus from potato plants

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Viral infection of potato (*Solanum tuberosum* L.) plants is very important due to their effect on potato yield and degeneration of seed tubers. This study aimed to eliminate potato virus Y (PVY) by tissue culture technique using different therapies, such as thermo-, chemo- and electrotherapies. Potato plants cv. Diamond cultivated at Faculty of Agriculture farm, Sohag University were tested by direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA) using antisera against PVY, Potato virus X (PVX) and Potato leaf roll virus (PLRV). The results indicated the occurrence of single and mixed infections of three viruses in potato plants. Survey results indicated highly distribution of PVY infected plants, which its yield was affected strongly. Tubers of potato plants which gave a positive reaction to PVY only were collected at harvest, stored in cold store room for three months and then planted in a greenhouse to obtain materials for virus elimination studies. Virus status *in vitro* plantlets was determined by double antibody enzyme linked immunosorbent assay (DAS-ELISA) and biologically test before and after treatments The two cycles of thermotherapy at approximately 37°C, during 40 and 30 days, resulted in 33.3 and 14.2% PVY elimination, respectively. Thus, these results indicated that thermotherapy is moderately efficient for viral eradication. Chemotherapy was undertaken with 20 mg l^{-1} Ribavirin (RBV); it is showed the medium rate of virus elimination (30.0 - 42.9% virus-free plantlets) when added to the tissue culture media. Several stems including axillary buds were excised from the potato plants grown for 45 days and electric-shocked treated. Stems were directly connected to the electrodes of power supply or indirectly by immersion of plant tissue in electrified water in a wide range of intensity-time. Axillary buds excised from electric-shocked stems or tissues immersed in electrified water, were transferred into the medium supplemented with or without ribavirin (RBV) to examine its combination effect. With an electric shock treatments alone (5, 10 and 15 mA electric current), the replicates become PVY-free in rates 53.8, 72.7 and 87.5%, respectively. Simultaneous, RBV and electric shock had higher efficiency for PVY elimination, reaching rates of healthy plantlets of 66.7% with 5 mA, 90.0% with 10 mA, and 100.0% with 15 mA.

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In this case, both electric shock and antiviral compound treatments in axillary buds from stem segment were successful in PVY-elimination; hence this treatment reported the most consistent. The resulted virus-free plantlets were further propagated via nodel cuttings in the medium without antiviral agent and then used as a mother plants for minituber production. Total yield was significantly higher in plants grown from virus-free seed minitubers when evaluated under field conditions in comparison with those grown from viral-infected tubers.

Key words: *Solanum tuberosum*, virus-free, thermotherapy chemotherapy, electrotherapy, PVY, ELISA and elimination

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most widely distributed crop in tropical and subtropical zones of the world and provide a stable food for millions of people. Potato production is threatened by different biotic agents such as bacteria, fungi and viruses. Viral infected plants characterized by a decrease in vigor, productivity, and resistance to other pathogens **(Sangar, *et al.*, 1988)**. The occurrence of potato viruses, including Potato virus Y (PVY), was observed in commercial fields **(Nascimento, *et al.*, 2003** and **Biswas, *et al.*, 2005)**, developing in a single and mixed infections. High virulence of these viruses, continuous introductions of the viruses through imported seeds and recurrent occurrence of the carrier/vector of some these viruses are the main impediments in directly controlling viral infections **(Ahmad and Ahmad, 1995)**. PVY is the type species of the genus *Potyvirus*, in the family *Potyviridae* **(Kitajima, *et al.*, 1997)**. Symptoms induced by PVY vary from an almost imperceptible mosaic up to severe necroses and premature death of plants, depending on cultivar and viral strain **(Barker, 1994; Sturz, *et al.*, 2000** and **Robert, *et al.*, 2000)**. Yield decrease resulting from PVY was found to range from 14 - 88% **(Spaar and Kleinhempel, 1986)**, while **De Bokx and Huttinga (1981)** pointed out that PVY reduced yield by 10–80% depending on the virus strain, potato cultivar and inoculation time. **Hane and Hamm, (1999)** presented that there are decrease in total and marketable yield by using PVY infected potato tubers in the field. Seedborne infected plants had fewer and smaller tubers than did

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plants grown from virus-free ones. **Whitworth *et. al.*, (2006)** found that PVY reduces yield in many cultivars. Seed tuber quality is an extremely important factor for potato yield, since it is a vegetative-propagated plant and viral disease agents are easily transmitted through the tubers **(Sturz, *et al.*, 2000** and **Nascimento, *et al.*, 2003)**. One of the most viable methods for obtaining virus-free stocks from propagative materials that comes from infected plants is viral eradication by using tissue culture techniques, aided or not by thermo- and/or chemotherapies **(Mellor and Stace-Smith, 1970** and **Awan *et al.*, 2007)**. These methodologies allow quick propagation of plant materials, producing healthy plants in a short period of time. Thermotherapy and meristem culture are commonly used for the production of virus-free stocks of potatoes **(Faccioli and Rubies-Autonell, 1982** and **Faccioli and Colombarini, 1996)**. **Klein and Livingston (1983)** described that, potato virus x (PVX) and potato virus Y (PVY) were eliminated by meristem tip culture and chemotherapy using ribavirin (1-β-D-ribofurasonyl-1,2,4 triazone-3-carboxamide), but the time required for regeneration of the tips was longer than the untreated controls. **Cassels and Long (1982)** and **Cassels (1987)**, reported that the inclusion of ribavirin in tissue culture media resulted in a high frequency of elimination of the potato virus complex X, Y, S and M. The application of electric pulses to eliminate viruses from plant tissue has recently received much attention. Using an electrotherapy **Quacquerelli, *et al.*, (1980)** obtained symptomless almond plants. Also, by using electrotherapy apparatus developed in Cuba (Patent Cuba 37/95 AO 1C/08 1524/97), treated garlic (*Allium sativum* L.), sugar cane (*Saccharum* spp.), banana (*Musa* sp.) and araceas (*Xanthosomas* and *Colocasia*) for potyvirus, Luteovirus, Cucumovirus and Carlavirus elimination, respectivily (cited from **Hernandez, *et al.*, (1999)**. For potato, **Yi, *et al.*, (2003)** and **Dhital, *et al.*, (2008)**, reported PVX, PVY and PLRV elimination in approximately 40% of regenerated plants. This study started because high yielding commercial varieties or lines shown durable resistance against potato viruses in Egypt are not available. So that, the study aimed to evaluate the efficiency of

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different therapies for PVY elimination in potato plants and find out the best one for eradication of virus and higher plant regeneration. Production of virus-free minitubers from mother plants for using it as potato tuber was also undertaken.

MATERIALS AND METHODS

Serological detiction of potato materials

A preliminary test was carried out with randomized sample leaves of 122 potato plants of cv. Diamond that exhibited viral infection symptoms and others showed no symptoms. The samples were obtained from the experiment cultivated on the 9th of October, 2005 in Faculty of Agriculture Farm, Sohag University. The experimental field were prepared and shaped to ridges 70 cm a part. Each experimental plots was 3×3.5 m (1/400 fed.) contained four ridges (70 cm wide and 3.5 m long). The seed tubers of potato cultivar were planted on the top of the ridges in hills at 25 cm apart. The normal culture procedures known for commercial potato production other than the applied treatments were performed. The samples were taken 35 days after planting and then plants covered with Agryl P17 Lutradur® (Lutravil Spinvil Comp., Liebigstr 2-8, Germany) for insect protection. The samples were indexed by direct antigen coating-Enzyme Linked Immunosorbent Assay (DAC-ELISA) test according to **Hobbs, *el al.*, 1987**, using antisera against PVY, PVX and PLRV each at the concentration of 1:1000 (v/v). The field plants having different types of infection were marked and the tuber yield was determined at harvest.

Obtaining PVY-infected potato stock

Tubers from 52 plants that reacted positively to PVY only were collected at harvest. The yield from each plant was placed in individual nylon mesh bags stored in storage room for three months and then planted in a greenhouse to obtain materials for further studies. After sprouting, plantlets were indexed by using PVY-antiserum to confirm the occurrence of single infection by PVY in the selected material. To be sure of freeing the plants from some other viruses, growing plants were tested biologically using specific indicator plants for each virus. Some leaves from each plant were

grinding in 0.01M pH 7.0 phosphate buffer and inoculating the following differential hosts: *Gomphrena globosa* L. and *Nicotiana glutinosa* (to detect, PVX and tobacco ring spot virus), *Datura metale* and *Solanum tuberosum* (for PVY and potato virus A), *Chenopodium amaranticolor* (for potato virus S and potato mop-top virus) and *Nicotiana tabacum* cvs. White Burley and Samsun (for tobacco ring virus and tobacco necrosis virus). By this method, plenty of infected tissues were available, since tissues were harvested after 45 days then used in subsequent tests.

Potato cultivation by tissue culture

For plant propagation, shoot inducing medium (SIM), shoot multiplication medium (SMM) and root inducing medium (RIM) were used as indicated in **Table (1)**, according to **Yi *et al.*, (2003)** and **Farzana (Shirin), *et al.*, (2007)**. Electric-treated axillary buds were removed from stem segments and sterilized by shaking them in 6% commercial bleach solution with several drops of Tween-20 for 10 min, followed by 60% ethanol for 5 min. The meristem tips (about 1.0 mm long) were excised from each bud with needles and razor blades, under a low-power microscope placed in sterile laminar-flow, then cultured in SIM at 25±2°C for 4-6 weeks. Differentiated shoots were excised then single node cuttings (1.0 cm length) were madel according to **Kluge *et al.*, 1990** and then transferred to SMM for shoot propagation and RIM for root formation. The pH of all media was adjusted at 6.0 by using 1 N NaOH, and immediately distributed into containers which were then sealed with aluminum foil and autoclaved at 121 °C for 20 min. Containers were incubated in a growth room, under a temperature regime of 25 ± 2°C and photoperiod cycle of 16/8 hrs as light/dark, provided by fluorescent tubes with light intensity of 2500 lux according to **Jayasree *et al.*, (2001)** for potato

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Table 1. The chemical components of media.

Components	Medium type**		
	SIM	SMM	RIM
Indolacetic acid (mg l^{-1})	0.1	0.0	0.1
Gibberelic acid(mg l^{-1})	0.2	0.2	0.2
Kinetin (mg l^{-1})	0.0	0.0	0.04
Ribavirin (mg l^{-1})	20.0	20.0	20.0
MS mix* (g l^{-1})	4.4	4.4	4.4
Sucrose (g l^{-1})	30.0	30.0	30.0
Agar (g l^{-1})	7.0	0.0	0.0

**SIM= shoot inducing medium. SMM= shoot multiplication medium. RIM=root inducing medium.

* MS basic salts according to **Murashige and Skoog, (1962).**

Treatments for virus elimination

All the samples used in this study were obtained from PVY-potato infected stock growing under greenhouse conditions for 45 days.

1- Thermotherapy

Thermotherapy was carried out in two cycles according to **Nascimento *et al.*, 2003**. Firstly, 14 PVY-infected stock plants, showing root growth and leaves, were submitted for 40 days to temperature of 37 ± 2°C and then viral indexed. By using single nodal cuttings grown in SMM and RIM maintained at 25 ± 2°C, several plantlets were subcultured from survived and remained infected plants after first cycle. Single node cuttings about 1.0 cm in length having one bud, were taken from the PVY-infected plants growing in greenhouse. These were sterilized as mentioned above. Explants were cultured individually and maintained at 25 ± 2°C under 16-hour photoperiod **(Awan, *et al.*, 2007)**. By using this method, a great number of infected plantlets, according to performed indexing were originated. Thirty-five plantlets reached between 8 and 10 cm, were utilized in the second cycle. During the second cycle, 35 plantlets established *in vitro* were transferred to growth chamber at 37 ± 2°C, whereas they remained for 30 days then indexed.

2- Chemotherapy

To analyze the separate effects of ribavirin on PVY elimination, the excised meristem-tips with no

electric shock were also cultivated in the SIM, SMM and RIM supplemented with 20 mg l^{-1} ribavirin. Ribavirin was filter-sterilized and added to culture midia.

3- Electrotherapy

The most vigorous stems were selected and treated with one of the followings: (1) the stem segments with approximately five axillary buds were directly connected to the electrodes for electric current intensity-time combinations: 5, 10 or 15 miliampers (mA) for 5 or 10 min, (2) stem segments with approximately 2 axillary buds were washed for 5 min in a detergent bath and then fixed in the electrophoresis chamber containing NaCl solution (1M) with the same range of intensity-time. Electricity was supplied by electrophoresis power supply (LABCONCO power supply 433-3240) as showed in **Figure 1**. After the treatment, the axillary buds were removed, surface-sterilized then meristem-tips were excised and cultured on SIM. For studying the combination effect of electrotherapy and chemotherapy, meristem-tips excised from electric shocked axillary buds were cultured in the medium supplemented with ribavirin (20 mg l^{-1}).

The parameters studied were % survival of plantlets after treatments; % virus elimination which was determined by DAS-ELISA and biologically test and therapy efficincy according to **Lozoya-Saldaña *et al.*, (1996)**.

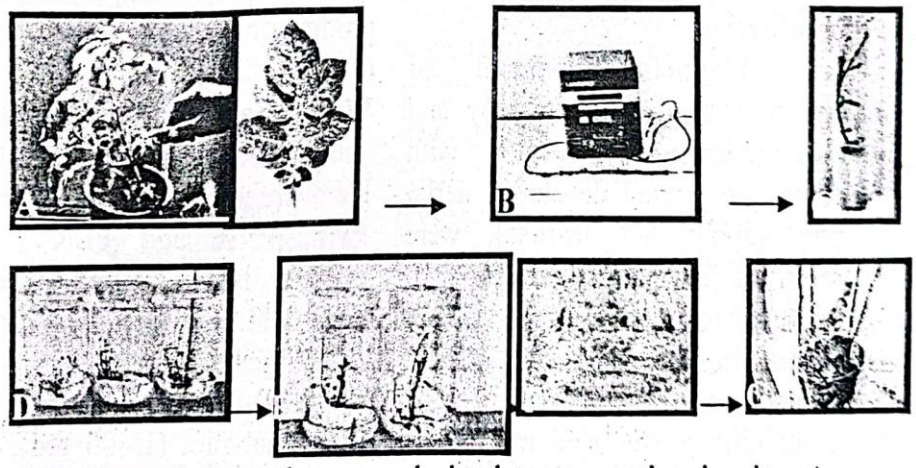

Fig. 1. Procedure of electrotherapy and plantlet regeneration in vitro A: cutting the stem of 45 day-old greenhouse grown potato plants

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infected with PVY, B: the stem segment treated with current intensity, C: induction of shoots from meristem-tips treated with current intensity, D: multiplication of shoots free from virus, E: multiplication of plantlets, F: roots induction and G: plantlets acclimatization.

PVY-detection

Detection of PVY was performed at the end of cultures with double antibody sandwich-enzyme linked immunosorbente assay (DAS-ELISA) according to **Clark and Adams (1977)**. Shoots (0.50 g) were homogenized in 1.0 ml of extraction buffer to give a constant of 1: 4. Tissue samples from healthy and infected mother or stock plants growing in the greenhouse were used as negative and positive controls.

Acclimatization and minituber production

Plantlets submitted to chemotherapy, thermotherapy and electrotherapy, regenerated with roots and a well developed aerial part (about 5-7 leaflets), were removed from the culture medium and their roots were washed-free of media in running water. These plants were acclimated in pots containing a sterilized mixture of soil, vermiculite and organic matter, in the rate of 2:2:1, and kept under a transparent polystyrene cover, for two weeks. The plants remained without incidence of direct sunlight during this period in greenhouse at 22-25°C. In the first weeks of acclimation, plants were irrigated daily with Hoagland nutrient solution **(Gold and Faccioli, 1972)**. After 3 weeks survivor plantlets were indexed serologically, and then used as mother plants. The virus-free plantlets can be transplanted at high density in pots filled with previous sterilized mixture in aphid-proof greenhouse to be used as mother plants for minituber production. Ten plantlets per pot (20 cm) were used.

Virus-free minitubers evaluation

The virus free minitubers, PVY infected potato tubers and PVY, PVX and PLRV mixed infected tubers were planted in open field on 10th of October, 2006 to compare among them. Each treatment was cultivated in individual plot (1/400 fed), which contain three replicates. Five plants from each plot were taken for measuring the vegetative growth

parameters; *i.e*, plant height (cm), number of tubers/plant, average weight of tuber and weight of tubers/plant.

RESULTS AND DISCISSION

Stock indexing and *in vitro* cultivation of PVY-infected plants

When the antisera against PVY, PVX and PLRV were utilized in the DAC-ELISA test, 79 and 43 plants, presented positive and negative reactions, respectively. The utilization of specific antisera allowed identification of individual and simultaneous presence in several combinations of the PVY, PVX and PLRV viruses **(Table 2)**. PVY was detected in 62 out of 122 indexed potato plants; of which 52 showed single infection and 10 mixed infections. On the other hand, the total yield was reduced especially on mixed infection plants compared to healthy ones **(Table, 2)**. From the tubers bearing from single PVY-infection, 33 PVY-infected plants were obtained in greenhouse; the other one plant was found have mixed infection by PVY and PVX according to biological test. The PVY infected plants were used in thermotherapy (14 plants) and electro- and chemotherapy (19 plants). This study supports the information on the legitimate use of specific antisera to obtain healthy material, just by verifying the presence or absence of viruses. Using the double antibody coating ELISA test for viral detection in potato, **Nascimento *et al.*, (2003)** observed the same sensibility when using individual or mixed antisera. The utilization of specific antisera allowed identification of individual and simultaneous presence, in several combinations, of the PVY, PVX and PLRV viruses in plants **(Table 2)**. On the other hand, biological test confirmed the ELISA results, except one plant which neglected because it reacted positively to PVX using *N. glutinosa*. So that, virologists are often used bioassay to test the presence of viruses because they tend to be fairly sensitive and indication of the presence of infection material.

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Table 2. Potato plants indexing by using specific antisera.

Viruses detected*	Reaction to antiserum**	No of plants positively reacted	Yield / plant	
			No of tubers	Weight of tubers
PVY	+	52	8.0	1.040
PVX	+	4	9.25	1.258
PLRV	+	11	6.5	0.806
PVY + PVX	+	3	5.3	0.634
PVY + PLRV	+	4	5.0	0.575
PVX + PLRV	+	2	5.0	0.555
PVY + PVX + PLRV	+	3	4.6	0.475
Control	-	0	12.4	2.292

*PVY= Potato virus Y; PVX= Potato virus X; PLRV= Potato leaf roll virus.
** + = positive reaction, - = negative reaction

Thermotherapy of plants multiplied *in vitro* by single node cuttings

From the 14 infected plants established in greenhouse and submitted to the first thermotherapy cycle (40 days), six plants were survived and indexed. The indexing showed that two survivors were virus-free, indicating that 33.3% efficiency was achieved for PVY elimination **(Figure, 2)**. 224 single nodel cuttings were obtained from infected plantlets grown on SIM and then propagated in RMM. From these, 166 plantlets were produced, corresponding to a 74.1% regeneration percentage. When 35 from 166 plantlets were submitted to the second thermotherapy cycle (30 days), only five produced PVY-free progenies, thus achieving a virus elimination index of 14.2% **(Figure, 2)**. This study indicated that, 37°C for periods ranging from 30 to 40 days are not efficient for viral eradication. So that, using temperature, can not be considered adequate method for virus elimination in potato plants cultivated *in vitro* from single nodel cuttings. These results are not agreement with that obtained by **Faccioli and Rubies-Autonell, 1982**; they eliminated PVX and PVY from *in vitro* potato plants by using 35°C for periods ranging from 16 to 27 days. On the other hand, **Nascimento, *et al.*, (2003)** reported free significant elimination of PVY. The percentage of virus elimination

with thermotherapy can be increased by the simultaneous adoption of other techniques **(Hollings, 1965, Stace-Smith and Mellor, 1968 and Slack, 1980)**. The effect of heat on viruses is not well understood but it believed to be effective in inhibiting viral replication and synthesis of movement proteins mainly by blocking transcription **(Mink, *et al.*, 1998)**. High temperature and the period to which the potato plants were exposed to it, did not positively influence their development. Contrary to these results **Kartha and Gamborg (1975)**, observed increasing in growth of cassava plant cultivated *in vitro*, when submitted to 35°C

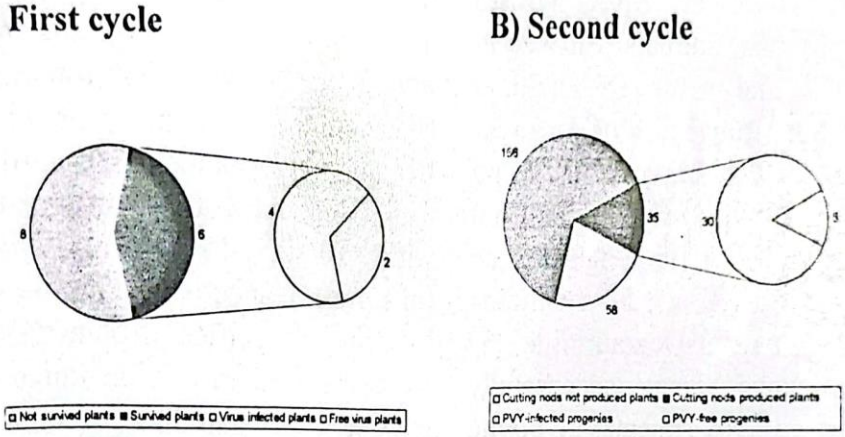

Fig. 2. Effect of thermotherapy on PVY elimination, A) first cycle, 2 plants from 6 survived plants were PVY-free, but 4 were PVY-infected plants. B) 35 plantlets resulted from infected cutting nodes taken after first cycle and then submitted to the second thermotherapy.

Chemotherapy

To investigate the effect of antiviral chemicals, ribavirin was used and the presence of virus was detected with DAS-ELISA test. The 20 mg l^{-1} ribavirin was moderately effective in elimination of PVY from all replicates. As shown in **Tables (3 and 4)**, about of 30.0% and 42.9%, were PVY-free, respectively. These percentages were decreased to 6.7% and 22.2 when ribavirin was absent. Results showed by **Simpkins *et al.*, (1981), Dodds *et al.*, (1989)** and **Griffiths *et al.*, (1990)** and indicated that addition of ribavirin (RBV) to the growth medium allowed satisfactory PVY elimination. The efficiency of RBV

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in the elimination of plant viruses is already well documented in the literature **(Simpkins *et al.*, 1981; Chen and Sherwood, 1991; Lizarraga *et al.*, 1991** and **Fletcher *et al.*, 1998)** and depends on the utilized concentration, host plant and type of infected tissue. This substance has a broad spectrum of action against DNA or RNA viruses infecting man, animals **(Sidwell *et al.*, 1972)** and plants **(De Fazio, *et al.*, 1980; Dawson and Lozoya-Saldana, 1984; Dawson, 1984** and **Hansen, 1984)**. *In vitro* culture and application of antiviral agents such as Ribavirin (RBV); 5-Azacitidina (5-AZA) and 3-Deazauridine (3-DZD), have been successfully utilized in experiments involving potato cultivars toward the elimination of PVX, PVY, PLRV, potato virus S (PVS) and Potato virus M (PVM) **(Brown *et al.*, 1988 and Kleinhempel *et al.*, 1990)**. This study indicated that PVY was not completely eliminated by the treatment of ribavirin alone and and showed severe growth abnormalities. So that, the present study suggested that the electrotherapy or combination treatment with other means might be necessary.

Electrotherapy

When stem segments were connected directly to electrodes, most replicates exposed to 5/5, 5/10 and 10/5 (mA/min) were confirmed as PVY-free (46.7, 57.1 and 64.7%, respectively). However, at 10/10, 15/5 and 15/10 (mA/min), these percent were increased to 73.3, 78.9 and 83.3%, respectively **(Table, 3)**. On the other hand, when the explants were fixed in the electrotherapy chamber, the percentages of virus-free plantlets was increased until to 83.3, 100 and 100% after treated with 10/10, 15/5 and 15/10 (mA/min), respectively **(Table, 4)**. Electrical properties of plant tissue are considered in a wide range of physiological studies **(Nelson, 1973)**. In this study, most replicates exposed directly to 15 mA were confirmed as PVY-free, which result 24.0% regeneration rate. The ratio of virus free reached to 83.3% when 15mA/10min was used **(Table, 3)**. This result was agree with **Startseva *et al.*, 1975**. So that, result of this study clearly indicated that, directly electricity treatment seemed to affect the elimination of virus effectively. On the other hand, the regeneration rate was severely retarded when explants were fixed in

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electrotherapy chamber (3.2%) **(Table, 4)**. It was also noticed that, the temperature of potato stems treated with electric current was raised. The temperature may be suppressed viral activity but not inhibit the plant metabolism. Also, they suggested that, stem sap pH may be changed after electric shock treatment, which is related to inhibition of virus replication **(Goldsworthy, 1987; Retivin and Opritov, 1992 and Helliot *et al.*, 2007)**.

Combination of chemotherapy and electrotherapy

It was assumed that combination treatment with electricity as well as ribavirin might be effective in the virus elimination. To eliminate PVY from the potato variety used in this study, some axillary buds from the segment directly treated by electricity were excised and cultured in the medium supplemented with ribavirin (20 mg l^{-1}). At the end of culture, it was confirmed that PVY was completely eliminated in all replicates treated by electric currents 15/5 and 15/10 (mA/min) then cultured in the medium with 20 mg l^{-1} ribavirin **(Table, 3)**. On the other hand, the better result was obtained when explants were fixed in electrotherapy chamber and then cultured on the same midium, whereas PVY was completely eliminated at low electric currents (10 mA/10min) **(Table, 4)**. This study confirmed that, PVY was eliminated in all replicates treated by electric current 15 mA for 5 min and cultured in the medium with 20 mg l^{-1} ribavirin. Virus elimination indices further support the use of electrotherapy, together with the addition of ribavirin to the growth medium, as the best treatment for virus elimination in potato **(Yi, *et al.*, 2003)**. Other authors such as **Griffiths *et al.*, (1990)**, observed similar results, virus concentration was reduced when the ribavirin was added to the medium and plants were submitted to thermotherapy. In present study, the plantlets freeing from virus were further propagated via nodel cutting in the medium without ribavirin because plants were propagated better without ribavirin. The technique of *in vitro* cultivation of single nodel cuttings aimed to propagation virus-free plantlets, since it allows the production of plants in approximately six weeks, as compared to the six months

required by meristem culture (**Griffiths *et al.*, 1990**).

Therapy efficiency (TE)

The greatest TE, 43.6%, was obtained in ribavirin-free medium, following exposure to 15 mA for 5 min. The resultant rate is 63.3% regenerated plantlets of which, 68.9% were virus-free. On the other hand, after ribavirin addition, the highest TE value was 25%, whereas regenerated plantlets were decreased to 31.3%, of which 80.0% were virus-free **(Table, 3)**. The TE values were decreased when explants were treated by fixation in the electrotherapy chamber. The maximum value reached to 20.7% after exposure to 10 mA for 5 min, then decreased to 3.2% following exposure to 15 mA for 10 min and cultured in medium supplemented with ribavirin, which decreased the regenerated plantlets to zero **(Table, 4)**. The TE was affected strongly when thermal treatment was used; it was 25% and 12.3% after exposure to the first and second cycles, respectively **(Figure, 1)**. Therapy efficiency TE was calculated by multiplying the percentage of regenerated plantlets X percentage of virus-free survivors. The data resulted from:

a) High regeneration but few virus-free plantlets (table 3, 45.5% and 6.7%, respectively).
b) Moderate behavior in both observations (table 3, 56.7% and 64.7%, respectively).
c) Low percentage of regeneration, although all resulting individual plantlets were virus-free (table 4, 3.2% and 100%, respectively).

Plant regeneration

To investigate the effect of thermal, chemical and electrical shock treatments on the generation rate of plantlets during the tissue culture, the regeneration and growing pattern of each regenerated plantlets were observed. As indicated in **Figure 2**, the rate of regeneration was retarded by thermal treatment. From 14 infected plants, which submitted to the first thermotherapy cycle, only six plants were survived. From 224 single nodel cuttings obtained, 166 plantlets were produced, corresponding to 74.1% regeneration. The rate of regeneration and growth of shoots were not greatly retarded by

treatment of ribavirin (20 mg l^{-1}) supplemented in the culture media. The regeneration rates were decreased after using ribavirin from 45.5 to 33.3% and from 30.0 to 26.0% **(Tables, 3 and 4)**, respectively. While electrical shock treatment slightly enhancement the regeneration rate until using 10mA/10min especially after directly treatment, then decreased after treatment with 15/5 and 15/10 (mA/min). On the other hand, electrical shocked plant materials and then cultured on ribavirin supplemented media, resulted in a serious reduction in the regeneration rate, especially when 15 mA current was used **(Tables, 3 and 4)**. According to **Wambugu *et al.*, (1985)**, ribavirin treatment reduced growth rate in culture treated with 20 mg l^{-1}, which showed severe growth abnormalities such as chlorosis, stunting, root inhibition and cause deformation. The effect of the electric shock treatment on the regeneration rate was differed when treated explants were cultured on medium supplemented with ribavirin. This result not agreement with some previous reports **(Goldsworthy, 1987)**. They reported that exposures to mild electric current, 5 to 10 mA for 5 min prior to *in vitro* culture, may improve regeneration of plant tissue. No morphological differences were observed between plants exposed to the different current treatments and controls.

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Table 3. Effect of directly electric current and ribavirin treatments on potato shoot-tip culture regeneration and PVY elimination.

Treatments		Regeneration		Virus-free plantlets		Therapy efficiency %*
Riba virin	Electric current (mA/min)	A	%	B	%	
With ribav irin	0/0	10/30	33.3	3/10	30.0	10.0
	5/5	12/35	34.3	8/12	66.7	22.9
	5/10	10/30	33.3	7/10	70.0	23.3
	10/5	10/32	31.3	8/10	80.0	25.0
	10/10	8/29	27.6	7/8	87.5	24.2
	15/5	7/28	25.0	7/7	100.0	25.0
	15/10	6/25	24.0	6/6	100.0	24.0
With out ribav irin	0/0	15/33	45.5	1/15	6.7	3.0
	5/5	15/31	48.4	7/15	46.7	22.6
	5/10	14/28	50.0	8/14	57.1	28.6
	10/5	17/30	56.7	11/17	64.7	36.7
	10/10	15/27	55.6	11/15	73.3	40.8
	15/5	19/30	63.3	13/19	68.9	43.6
	15/10	6/25	24.0	5/6	83.3	20.0

A= Plantlets regenerated / number of treated buds cultured.

B= PVY-free plantlets / plantlets regenerated.

* Therapy efficiency (TE) = A% x B% according to **Lozoya-Saldaña *et al.*, (1996).**

Acclimatization and minituber production

After 3 weeks from beginning of acclimation, survivor plantlets were indexed serologically, and then used as a mother plants, whereas 10 plantlets per pot were used. During 70-80 days growing period, each pot can produce about 45 minitubers and a weight range of 3-7g per tuber.

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Table 4. Effect of indirectly electric current and ribavirin treatments on potato shoot-tip culture regeneration and PVY elimination.

Treatments		Regeneration		Virus-free plantlets		Therapy efficiency %*
Ribavirin	Electric current (mA/min)	A	%	B	%	
With ribavirin	0/0	7/27	26.0	3/7	42.9	11.2
	5/5	9/33	27.3	6/9	66.7	18.2
	5/10	8/30	27.0	6/8	75.0	20.3
	10/5	7/29	24.1	6/7	85.7	20.7
	10/10	5/27	18.5	5/5	100.0	18.5
	15/5	4/30	13.3	4/4	100.0	13.3
	15/10	0/31	0.0	0/0	0.0	0.0
Without ribavirin	0/0	9/30	30.0	2/9	22.2	6.7
	5/5	9/29	31.1	5/9	55.6	17.2
	5/10	10/32	31.3	6/10	60.0	18.8
	10/5	8/29	27.6	6/8	75.0	20.7
	10/10	6/29	20.7	5/6	83.3	17.2
	15/5	5/28	17.9	5/5	100.0	17.9
	15/10	1/31	3.2	1/1	100.0	3.2

A= Plantlets regenerated / number of treated buds cultured.

B= PVY-free plantlets / plantlets regenerated.

*Therapy efficiency (TE) = A% x B% according to **Lozoya-Saldaña *et al.*, (1996).**

Virus-free minitubers evaluation

The results in **Table (5)** presented that the virus-free minitubers was the best than other infected seed tubers concerning for plant height, number of tubers/plant, average weight of tuber and also, total yield/plant characters. Potential losses are even greater in plants grown from PVY-infected tubers when compared with those grown from virus-free minitubere seeds. The virus-free minitubers gave the highest value than other infected tubers. The infected plant with PVY was milder symptoms than the infected plant with mixed viruses. The obtained results agreement with many workers such as **Spaar and Kleinhempel, 1986; De Bokx and Huttinga 1981; Sangar, *et al.*, 1988; Hane, and Hamm, 1999 and Whitworth *et. al.*, 2006.**

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Table 5. Evaluation of plants resulted from virus free minitubers compared with virus infected plants.

Potato seed sources	Plant height (cm)	Number of tubers/plant	Average of weight tuber (g)	Total yield/plant (g)
Virus-free minitubers	41.66 a	10.67 a	161.6 a	1616.7 a
Tubers infected with PVY	38.33 b	8.67 b	128.3 b	1216.6 b
Tubers infected with mix of PVY, PVX and PLRV	37.30 c	7.30 c	110.0 c	866.7 c
L.S.D at 0.05	0.75	1.18	17.1	173.1

According to these results, efficiency of electrotherapy combined with ribavirin for eradication of potato viruses was higher. This technique must be combined with strong certification, which result plant materials for using to boost production of virus-free seed tubers. Also, using single node cuttings method especially in presence of ribavirin treatment was found to be technically easier and simpler than meristem culture. This study also demonstrated the usefulness of biologically test together with ELISA for direct detection of PVY from all sambles. Growers in Egypt, used cutting seed tuber to pieces in-season of cultivation for potato production. At present, a tendency towards of a whole-tuber planting by using small size of seed tubers was achieved. This procedure can be prevented some pathogen (such as ring rot, bacterial wilt, PVX, potato spindle tuber viroid) transmitted by tuber-cutting. Also, due to planting whole small-size tuber for production, the labor for tuber-cutting could be saved. So that, development of seed potato production system characteristic of minituber multiplication must be expansion for virus-free seed potato.

REFERENCES

Ahmad, M. and Ahmad, W. (1995). Detection of major potato virases from different potato growing localities of Punjap. ***Natl Sem. On "Research and development in potato production in Pakistan" PSPDP/APC, Islamabad, Pakistan.***

Awan, A. R.; Mughal, S. M.; Iftikhar, Y. and Khan, H. Z. (2007). *In vitro* elimination of potato leaf roll polerovirus from potato varieties. ***Eur. J. of Scientific Res., 18(1): 155-164.***

Barker, H. (1994). Incidente of potato virus Y infection in seed and tubers of the potato cv. Recard. ***Ann. Appl. Biol., 124:179-183.***

Biswas, M.K.; De, B.K. and Nath, P.S. (2005). Rate of spread of PVX, PVY and PLRV diseases to potato varieties. ***Ann. Pl. Protec. Sci., 13(1):165-178.***

Brown, C. R.; Kwiatkowski, S.; Martin, M. V. and Thomas, P.E. (1988). Eradication of PVY from potato clones through excision of meristems from *in vitro*, heat treated shoot tips. ***American Potato Journal, 65: 633-638.***

Cassels, A.C. (1987). *In vitro* induction of virus-free potatoes by chemotherapy, P.40-50. ***In: Y.P.S.Bajaj (Ed), Biotechnology in agriculture and forestly: 3. Potato. Springer-Verlage.***

Cassels, A.C. and Long, R.D. (1982). The elimination of potato virus X, Y and M in meristem and explant cultures of potato in the presence of virazole. ***Potato Research, 25:165-173.***

Clark, M. F. and Adams, A. N. (1977). Characterization of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant virus. ***J. Gen. Virol., 34: 475-483.***

Chen, W.Q. and Sherwood, J. L. (1991). Evaluation of tip culture, thermotherapy and chemotherapy for elimination of peanut mottle virus from *Arachis hypogaea*. ***J. of Phytopathology, 132: 230-236.***

Dawson, W.O. (1984). Effects of animal antiviral chemicals on plant viruses. ***Phytopathology, 74: 211-213.***

Dawson, W.O. and Lozoya-Saldana, H. (1984). Examination of the mode of action of ribavirin against tobacco mosaic virus. ***Intervirology, 22:77-84.***

De Bokx, J. A. and Huttingah, D. (1981): Potato virus Y. CMI/AAB Descriptions of Plant Viruses No 242. ***Common wealth Agricultural Bureaux/Association of Applied Biologists.***

De Fazio, G.; Caner, J. and Vicente, M. (1980). Effect of

Egyptian J. Virol. 5, 561-586 (2008)

virazole (Ribavirin) on tomato spotted wilt virus in two systemic hosts, tomato and tobacco. *Archives of virology, 93:305-309.*

Dhital, S. P.; Lim, H. T. and Sharma, B. P. (2008). Electrotherapy and chemotherapy for eliminating double-infected potato virus (PLRV and PVY) from *in vitro* plantlets of potato (*Solanun tuberosum* L.). ***Hort. Environ.***

Dodds, J.H.; Lizarraga, R; Griffiths, H. and Slack, S.A. (1989). Methods of virus eradication. In: Planning Conference on Control of Virus and Virus-Link Diseases of Potato and Sweet potato, 3., Lima. ***Report. Lima: International Potato Center, 228p.***

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 Research, 39:129-140.***

Faccioli, G. and Rubies-Autonell, C. (1982). PVX and PVY distribution in potato meristem tips and their eradication by the use of thermotherapy and meristem-tip culture. ***Phytopathologische Zeitchrift, 103:66-76.***

Farzana (Shirin); Hossain, M.; Kabir, M.F.; Roy, M. and Sarker, S.R. (2007). Callus induction and plant regeneration from internodal and leaf explants of four potato (*Solanum tuberosum* L.) cultivars. ***World J. of Agric. Sci., 3(1): 1-6.***

Fletcher, P.J.; Fletcher, J.D. and Cross, R.J. (1998). Potato germplasm: in vitro storage and virus reduction. ***New Zeland Journal of Crop and Horticultural Science, 26: 249-252.***

Gold, A.H. and Faccioli, G. (1972). Comparative effect of curly top infection and ethylene treatment on tomatoes and other plants. ***Phytopathologia Mediterranea, 11:145-153.***

Goldsworthy, A. (1987). Electrical stimulation of tissue culture growth and morphogenesis. ***Agricell Repory, 8:14.***

Griffiths, H.M.; Slack, S.A. and Dodds, J.H. (1990). Effect of chemical and heat therapy on virus concentrations in *in vitro* potato plantlets. ***Canadian Journal of Botanic, 68: 1515-1521.***

Egyptian J. Virol. 5, 561-586 (2008)

Hane, D. C., and Hamm, P. B (1999). Effects of seedborne potato virus Y infection in two potato cultivars expressing mild disease symptoms. ***Plant Dis. 83:43-45.***

Hansen, A.J. (1984). Effect of ribavirin on green ring mottle causal agent and necrotic ringspot virus in *Prunus* species. ***Plant Diseases, 68:216-218.***

Helliot, B.; Panis, B.; Hernandez, R.; Swennen, R; Lopoivre, P. and Frison, E. (2007). Development of *in vitro* techniques for the elimination of cucumber mosaic virus from banana (*Musa* spp.). ***http://www.fao.org/docrep/007/ae216e/ae216eoh.htm***

Hernandez, R.; Fontanella, J.; Noa, J.C.; Pichardo, T.; Igarza,Y.; Cardenas, H.; and Manso, R. (1999). Electrotherapy: a noval method for eliminating viruses from garlic (*Allium sativum* L.). ***Horticulura Argentina, 16(40/41):68-71.***

Hobbs, H.A.; Reddy, D.V.R.; Rajeshwar, R.; Reddy, A.S. (1987). Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. ***Plant Disease, 71: 747-749.***

Hollings, M. (1965). Disease control through virus-free stock. ***Annual Review of Phytopathology, 3: 367-396.***

Jayasree, T.; Pavan, U.; Ramesh, M.; Rao, A.V.; Reddy, J.M. and Sadanandam, A. (2001). Somatic embryogenesis from leaf cultures of potato. ***Plant Cell, Tissue and Organ Culture, 64: 13-17.***

Kartha, K.K. and Gamborg, O.L. (1975). Elimination of cassava mosaic disease by meristem culture. ***Phytopathology, 65: 826-828.***

Kitajima, E.W.; De Avila, A.C. and Resende, R.O. (1997). Taxonomia de vírus de plantas. ***Fitopatologia Brasileira, 22: 5-24.***

Klein,R.E. and Livingston,C.H. (1983). Eradication of potato viruses X and S from potato shoot tip cultures with ribavirin. ***Phytopathology, 73:1049-1050.***

Kleinhempel, D.; Schenk, G.; Bittner, H.; Gase, G. and Kurzinger, B. (1990). Determination of virus resistance under *in vitro* conditions. ***European Association for Potato Research, 5: 341-342.***

Egyptian J. Virol. 5, 561-586 (2008)

Kluge, S.; Moschke, M. and Schulze S. (1990). Studies of the use of antiphytoviral compounds in potato stem cutting cultures considering the quantitative virus resistance of cultivars. ***Arch. Phytopathol. Pflanzenschutz, Berlin, 26(4): 353-358.***

Lizarraga, R.; Panta A.; Jayasinghe R. and Dodds J. (1991). Tissue culture for elimination of pathogens. ***Lima: International Potato Center, 21p. (Research Guide, 3).***

Lozoya-Saldaña, H.; Abelló, F. and García de la R. (1996). Electrotherapy and shoot tip culture eliminate potato virus x in potatoes. ***Am. Potato J., 73: 149-154.***

Mellor, F.C. and Stace-Smith, R. (1970). Virus strain differences in eradication of potato virus X and S. ***Phytopathology, 60: 1587-1590.***

Mink, G. I.; Wample, R. and Howell, W. E. (1998). Heat treatment of perennial plants to eliminate phytoplasmas, viruses and viroids while maintaining plant survival. ***In "plant virus disease control" (eds: A. Hadidi, R. K. Ketharpal and H. Koganezawa), pp 249-300.***

Murashige, R. and Skoog, F. (1962). A revised medium for rapid growth and biossay with tobacco tissue culture. ***Physiologia Plantarum, 15: 473-497.***

Nascimento, L.C.; Pio-Riberio,G.; L. Willadino and G.P. Andrade (2003). Stock indexing and potato virus Y elimination from potato plants cultivated ***in vitro. Scientia Agricola, 60(3):525-530.***

Nelson,S.O. (1973). Electrical properties of agricultural products (a critical review). Special Publication SP-05-73. ***American Society of Agricultural Engineers. St. Joseph, Mich USA. 21pp.***

Quacquerelli, A. (1980). The use of electric current for obtaining mosaic free almonds. ***Acta Phytopathol. Acad. Sci. Hungaricae, 15:251-255.***

Retivin,V.G. and Opritov,V.A. (1992). Estimation of cold resistance of higher plants from electrophysiological analysis of their excitability. ***Sov. Pl. Physiol., 39:821-825.***

Robert,Y.; Trefor Woodford, J.A. and Ducray-Bourdin, D.G. (2000). Some epidemiological approches to the control of aphid-borne virus

diseases in seed potato crop in northern Europe. *Virus Research, 71:33-47.*

Sangar, R.B.S.; Agrawal, H.O. and Nagaich, B.B. (1988). Studies on the translocation of potato viruses X and Y in potatoes. *Indian Phytopathology, 41: 327-331.*

Sidwell, R.W.; Huffman, J.H.; Khare, G.P.; Allen, L.B.; Witkowski, J.T. and Robins, R.K. (1972). Broad spectrum antiviral activity of virazole 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxiamide. *Science,177:705-706.*

Simpkins, I.; Walkey, D.G.A and Neely, H.A. (1981). Chemical supression of virus in cultured plant tissues. *Annals of Applied Biology, 99: 161-169.*

Slack, S.A. (1980). Pathogen-free plants by meristem-tip culture. *Plant Disease, 64: 14-17.*

Spaar D. and Kleinhempel H., (1986). Borba s virusnimi baljeznjami rastenij. *Agropromizdat, Moskva, pp. 479.*

Stace-Smith, R. and Mellor, F.C. (1968). Eradication of potato viruses X and S by thermotherapy and axilary bud culture. *Phytopathology, 58: 199-203.*

Startseva, A.V.; Burtaeva, T.E. and Ruban, N.F. (1975). Effect of a weal electric current on the state of water in wheat leaves. *Sov. Pl. Physiol., 22:746-749.*

Sturz, A.V.; Stewart, J.G.; McRae, K.B.; Diamond, J.F.; Lu, X. and Singh, R.P. (2000). Assessment of the importance of seed cutting, in-season cultivation and the passage of row equipment in the spread of PVY^0 in potatoes. *Can. J. Plant Pathol., 22:166-173.*

Wambugu, F.M.; Secor, G.A. and Gudmestad, N.C. (1985). Eradication of potato virus Y and S from potato by chemotherapy of cultured axillary bud tips. *Amer. Potato J., 62:667-672.*

Whitworth, J. L., Nolte, P., McIntosh, C., and Davidson, R. (2006). Effect of Potato virus Y on yield of three potato cultivars grown under different nitrogen levels. *Plant Dis. 90:73-76.*

Yi, J.Y.; Seo, H.W.; Choi,Y.M. and Park,Y.E. (2003). Ribavirin, electric current and shoot-tip culture to eliminate several potato viruses. *J. Plant Biotechnology, 5(2):101-105.*