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Original Paper

Evaluation of some therapies and meristem culture to eliminate Potato Y potyvirus from infected potato plants

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Abstract Meristems (length 100, 200, 300 μm) were excised from infected Potato plants of Binella and Burren cultivars and cultured on solidified MS medium containing 30 gl^{-1} sucrose, 5 mgl^{-1} Ascorbic acid, 5 mgl^{-1} Pyridoxine, 5 mgl^{-1} Nicotinic acid, 5 mgl^{-1} Thiamine, 200 mgl^{-1} Inositole, 2 mgl^{-1} GA3, and 0.2 mgl^{-1} kinetin. Virus status of *in vitro* plantlets was determined by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). Results showed that the highest rate of virus-free plants was obtained by using explants 100 μm in length. The rate of PVY elimination was improved after treatment by thermotherapy at approximately 37±1°C for 40 days, (81%) in Binella and (75%) in Burren. Chemotherapy was undertaken with (10–20–30) mgl^{-1} of ribavirin (RBV), The highest percentage of virus free plantlets 87% in Binella and 82% in Burren were obtained from the ribavirin's concentration (20 mgl^{-1}) combined with meristem-tips (100 μm) in length. Severe growth abnormalities were observed on medium containing high concentration of RBV (30 mgl^{-1}). Finally, the highest percentage virus free plantlets of PVY (93% in Binella and 87% in Burren) were obtained from meristem-tips 100 μm in length excised after electric treatments (15 mA/10 min).

Key words: Potato, meristem culture, electrotherapy, thermotherapy, chemotherapy.

Potato (*Solanum tuberosum* L.) is a vegetable crop of major economic importance in the world. It is most productive, common and multiuse horticultural vegetable crop. Potato produces more protein and calories per unit area, per unit time and per unit of water than any other major food plant (Villamayor 1984). Potato is used worldwide for human and animal consumption, and as raw material for starch and alcohol production (Carputo et al. 2005).

In Syria, potato is one of the most important vegetable crops. The total potato harvested area reached about 34,855 ha, which produced more than 700,000 tons in 2009 (Annual Statistical Abstract issued by the Central Bureau of Statistics in Damascus, 2009).

More than 30 viruses infect potatoes of which actually five or six are important, the losses due to viruses infection are usually quantitative but some cause qualitative as well (Khurana 1992). The Potato Virus Y (PVY) is one of the most common and destructive viruses found in potato (Singh et al. 2008). It is the type species of the genus Potyvirus, family Potyviridae.

Potatoes are propagated vegetatively which allows for the passage of viral diseases from one generation to the next, making it possible for entire clonally populations to become infected with the same pathogen. This makes virus eradication an extremely valuable management tool in potato production (Kassins et al. 1957). Plant tissue culture techniques for elimination of viral infection in plants were applied by (Morel and Martin 1952); they cultured meristem tips excised from infected Dahlia and obtained disease-free plants. The traditional techniques applied to clean viral diseases in plants, i.e. Meristems culture, fails to produce enough quantities of clean material in some species. The successful conventional methods of virus eradication from plants by meristem culture is low and less efficient in producing virus-free plants (Hidayat and Erkol 2004; Nasir et al. 2010; Meybodi et al. 2011). Alternative, new techniques of thermotherapy, chemotherapy and electrotherapy have become efficient tools to overcome this problem (Black 1971; Bayaty et al. 2011). In potato (Yi et al. 2003) and (Dhital et al. 2008) were reported that electrotherapy with meristem culture used for PVX, PVY and PLRV elimination.

This study aimed to evaluate the efficiency of three different therapies (thermo-, chemo- and electrotherapies) with meristem culture for PVY elimination in potato plants and to find out the most appropriate one for eradication of virus with the higher plant regeneration.

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The biological material used in this study was represented by two potato cultivars: Burren and Binella (class B). Those cultivars differ morphologically, Binella has a fairly good resistance against virus Y, but Burren has a medium resistance to this virus.

Tubers were washed under running water, to remove all impurities, and then treated with Gibberellic acid (4 PPM of GA3) for 15 min in order to break the dormancy phase. Tubers were cultured excised and cultured in pots contained peat-moss. After 45 days, rose-ends were removed and indexed by DAC-ELISA (Direct Antigen Coating-Enzyme Linked Immunosorbent Assay) test according to (Hobbs et al. 1987), using antiserum against PVY, each at the concentration of 1 : 1000 (v/v), in order to insure the dealing with infected plants.

Rose-ends were treated with alcohol (70%) for 60s, immerged afterthat in sodium hypochlorite (3%) for 10 min, and washed 3 times with sterilized distilled water for 5 min to eliminate the excess of hypochlorite.

Shoot tips 0.5–1 cm in length were removed and cultured in aseptic conditions on initial culture medium, which contains MS salts, 30 $g l^{-1}$ sucrose and 7 $g l^{-1}$ Agar. The excised explants were incubated in a growth room, under a temperature regime of 25±2°C and photoperiod cycle of 16/8 h as light/dark, provided by fluorescent tubes with light intensity of 2.5 $\mu mol \cdot m^{-2} S^{-1}$ according to (Jayasree et al. 2001). Shoots obtained were cut and multiplied on the same medium one time each 20 days, in order to obtain enough material for therapies investigations.

Three lengths of Meristem (100–200–300 μm) were excised from virus-infected plants under a binocular dissection microscope and cultured under aseptic conditions on medium, which contains mineral salts of MS Murashige and Skoog (1962), supplemented with 2 $mg l^{-1}$ glycine, 5 $mg l^{-1}$ nicotinic acid, 5 $mg l^{-1}$ pyridoxine, 5 $mg l^{-1}$ thiamine, ascorbic acid 5 $mg l^{-1}$, inositole 200 $mg l^{-1}$, gibberellic acid 2 $mg l^{-1}$, 0.2 $mg l^{-1}$ kinetin, and 3% sucrose. The medium was solidified with agar (0.6%) and then sterilized by the autoclave (121°C for 15 min). The cultured meristems were transferred every 20 days to a new initial medium.

In vitro virus-infected potato plants, showing growth and leaves from two cultivars, were submitted for 40 days to temperature of 37±2°C under a high intensity continued light (5 $\mu m \cdot m^{-2} S^{-1}$). Apical meristem tips in different length (100–200–300 μm) from treated and non treated shoots were excised in sterile conditions and transferred to glass tubes with 12.5 ml of meristem medium. Sixteen explants from each cultivar were used for each treatment. Experiment was repeated twice. The total number of explants cultured was 3 (meristem length)×2 (cultivars)×2 (repetition)×16 (explants number)=192.

Different Ribavirin concentrations (10–20–30 $mg l^{-1}$) were added to the meristem culture media. The apical meristem tips in different length (100–200–300 μm) from two cultivars were excised in sterile conditions and transferred to glass tubes with 12.5 ml of meristem medium with Ribavirin. The control of this experiment was the same control of the thermotherapy experiment. Sixteen explants from each cultivar were used for each treatment. Experiment was repeated twice. The total number of explants cultured was 3 (meristem length)×3 (conditions)×2 (cultivars)×2 (repetition)×16 (explants number)=576 explants.

In vitro virus-infected plantlets with good growth (12–15 cm) fixed in the electrophoresis chamber containing NaCl solution (1 N), then the electric current was applied, the amount of current and time duration applied were: 15 miliampers (mA) for 5 and 10 min. After the treatment, the apical meristem tips were excised in different lengths (100–200–300 μm) under sterile conditions and transferred to glass tubes with 12.5 ml of meristem medium. Sixteen explants from each cultivar were used for each treatment. Experiment was repeated twice. The total number of explants cultured was 3×2×2×2×16=384 explants. Multiplication phase aimed to produce a good number of good shoots. For that, shoots have been moved from the previous phases (meristem medium) to the propagation medium containing mineral salts of MS medium, supplemented with 30 $g l^{-1}$ sucrose and 0.6% agar. Explants were transferred to the same medium, one time each 20 days, in order to obtain plant material sufficient for the other experiments.

Detection of PVY was performed by DAS-ELISA (Double Antibody Sandwich-Enzyme linked Immunosorbent Assay) according to (Clark and Adams 1977).

Several parameters were recorded, infected percentage, survival percentage, growth %, elongation rate, virus free plant %. All recorded data were the mean of two experiments. All statistical interpretations were done using SPSS Program by Completely Randomized Block Design at 1%.

One week after of the initial culture, observed results illustrated that the infection percentage was very low (less than 5%), no browning explants was observed, and growth percentage was very high (more than 95%) in explants cultured from two Potato cultivars.

After 30 days of culture, shoot tips burst and developed to give one shoot 7–10 cm in length. Plant samples were transferred from initial culture stage to the propagation medium to increase the number of shoots which came from rose-ends culture of the tubers of two potatoes varieties (Binella and Burren). All obtained explants tested by ELISA were infected by PVY.

The meristems in different lengths (100–200–300 μm) were excised from *in vitro* shoots obtained from initial

culture of PVY infected two cultivars of potato (Binella and Burren).

Results showed that the meristem's (100 μm) in length gave the highest rate of virus-free plants (56% in Burren and 69% in Binella), while the length (300 μm) gave the lowest rate (19% Burren and 25% Binella) (Table 1). Responses of Potato genotypes to therapies were significantly different. Our results agreed with the observations obtained by (Hidayat and Erkol 2004) in the production of virus-free plants from several cultivars of potato (Granola, Pasinler 92 and Caspar). The % of virus free plants obtained were 40% in Granola, 41.6% in Pasinler 92, and 33.3% in Caspar respectively. Similar results were reported by (Nasir et al. 2010), when meristem-tips were excised and cultured alone without any treatment from several cultivar of potato (Desiree, Cardinal, Diamont and Sante).

Elongation rate was obtained by using meristems (300 μm) in length (9.90 cm) in Burren and (9.6 cm) in Binella after 60 days while the use of explants (100 μm) in length gave the lowest elongation rate (5.4 cm) in Burren and (4.9 cm) in Binella (Table 1). But the risk of infection by the virus also increases with explants size.

This failure to invade the meristem is due to: High auxin concentration in meristematic cells, competition for nutrients enzymes for virus replication, Active metabolic process which is not suitable for virus multiplication, action of growth regulators (Cytokinins), presence of inhibitors (Phenolamines), and metabolic disruption of enzymes necessary for viral replication, RNA degradation (Wang et al. 1980). The efficiency of therapies depends greatly on kind of virus, the genetic background of a potato cultivar and the virus concentration in the parent stock (Vinayak et al. 1999).

Similar results were reported by the International Potato Center (CIP) in (1993) which showed that Binella has a Medium to high resistance to PVY and Burren has fairly good resistance to PVY. Long and Cassells (1984) reported that the larger meristem length gave the higher percentage of survival and shoot development than smaller ones.

The indexing Results showed that the highest rate of virus-free plants was achieved by using meristem 100 μm in length, after heat-treatment (75% in Burren and 81% in Binella) while in meristem 300 μm in length, the lowest rate of free-virus plants was obtained (56% in Burren and 69% in Binella). (Table 2).

Our results after 40 days did not agree with that obtained by (Faccioli and Rubies-Autonell 1982); where they eliminated PVY from *in vitro* potato plants by using 35°C for periods ranging from 16 to 27 days. So the duration of heat treatment should be decided judiciously, the excessive exposure to high temperature may adversely affect the plant tissues. Similar results were reported by (Luciana et al. 1993) when they submitted the infected *in vitro* plants for 40 days to temperature of 37±2°C.

The basic principle of heat eradication of viruses is that

Table 1. Effect of meristem's length on the survival, growth and % of infected plants of *in vitro* Potato plantlets of Binella and Burren.

Meristem's length (μm)	Meristem survival rate (%)		Mean shoot length (cm) after 60 days		Infected plants %	
	Burren	Binella	Burren	Binella	Burren	Binella
100	88^{c}	86^{d}	5.40^{c}	4.90^{c}	44^{c}	31^{a}
200	100^{a}	94^{b}	7.70^{b}	6.60^{bc}	56^{d}	38^{b}
300	100^{a}	100^{a}	9.90^{a}	9.60^{a}	81^{f}	75^{e}
LSD=	1.59		1.41		2.044	

Three lengths of Meristem (100–200–300 μm) were excised from virus-infected plants under a binocular dissection microscope and cultured under aseptic conditions on meristem medium, The cultured meristems were transferred every 20 days to a new initial medium.Responses with the same letter are not significantly different at $p=0.01$ by ANOVA test.

Table 2. Effect of Thermotherapy on the survival, growth and % of infected plants of *in vitro* plantlets of Binella and Burren.

Treatment	Meristem's length (μm)	Meristem survival rate %		Mean shoot length (cm) after 60 days		Infected plants %	
		Binella	Burren	Binella	Burren	Binella	Burren
Thermotherapy	100	88^{c}	88^{c}	5.20^{cd}	5.60^{cd}	19^{a}	25^{b}
	200	94^{b}	94^{b}	7.70^{b}	8.00^{b}	25^{b}	31^{c}
	300	100^{a}	100^{a}	9.80^{a}	10.40^{a}	31^{c}	44^{e}
Control	100	86^{d}	88^{c}	4.90^{d}	5.40^{cd}	31^{c}	44^{e}
	200	94^{b}	100^{a}	6.60^{bc}	7.70^{b}	38^{d}	56^{f}
	300	100^{a}	100^{a}	9.60^{a}	9.90^{a}	75^{g}	81^{h}
LSD=		1.59		1.41		2.044	

In vitro virus-infected potato plants of cv Binella and cv Burren were submitted. for 40 days to temperature of 37±2°C under a high intensity continued light (5 μm·m^{-2}S^{-1}). Apical meristem tips in different length (100–200–300 μm) were excised in sterile conditions and transferred to glass tubes with 12.5 ml of meristem medium.Responses with the same letter are not significantly different at $p=0.01$ by ANOVA test.

at the temperatures higher than normal many viruses in plant tissue are partially or completely inactivated with little or no injury to the host tissues (Baker 1962). At elevated temperatures, synthesis of viral ssRNA and dsRNA is disrupted. It has also been suggested that coat proteins, which are involved in cell-to-cell movement of viruses through plasmodesmata and long-distance movement through the plant vascular system, may also play a role in the effectiveness of heat therapy for virus elimination (Kassanis 1957; Quak 1977).

The lowest elongation was noticed by using meristem (100 μm) in length (5.6 cm) in Burren and (5.2 cm) in Binella, but the highest elongation was achieved by using the length (300 μm) (10.4 cm) in Burren and (9.8 cm) in Binella.(Table 2).

The highest numbers of virus-free plantlets were obtained from the Ribavirin's concentration (30 mg l^{-1}) combined with the meristem 100 μm in length (87% in Binella and 82% in Burren), and similar percentages (87% in Binella and 81% in Burren) were achieved when the Ribavirin's concentration 20 mg l^{-1} was added to the meristem's medium and combined with meristem 100 μm in length. These percentages were decreased to 50% in Burren and 56% in Binella when ribavirin's concentration (10 mg l^{-1}) was added with the meristem 300 μm in length (Table 3). Similar results was reported by (Gudmestad et al. 1985) when he added the ribavirin (20 mg l^{-1}) to the meristem's medium. These results did not agree with that recorded by (Gianni 2002) when he added the Ribavirin (50 mg l^{-1}) to achieve the highest percentage of free-virus plants.

Although the same percentage of free-virus plants was obtained by adding Ribavirin concentrations (20–30 mg l^{-1}), but the shoots grown in the meristem's medium contained ribavirin (30 mg l^{-1}) showed yellowish color and weak growth due to the fact that ribavirin may cause death of some meristems or slow down the cellular metabolic processes (Faccioli 2001) but the plantlets obtained from ribavirin-supplemented media (30 mg l^{-1}) were later developed normally, when they were taken out of the concentrations of many antiviral chemicals that are required during chemotherapy to inhibit virus multiplication. Those concentrations are very close to the concentrations which are toxic to host plant.

This substance (Ribavirin) has a broad spectrum of action against DNA or RNA viruses infecting human, animals (Sidwell et al. 1972) and plants (De Fazio et al. 1980; Dawson and Lozoya-Saldana 1984; Dawson 1984) also ribavirin inhibits RNA-capping enzyme leading to inefficient translation of mRNA.

The highest elongation achieved when we used the size (300 μm) in the lowest concentration of ribavirin (10 mg l^{-1}) (7.8 cm) in Burren and (6.5 cm) in Binella, while the usage of the size (100 μm) in the highest concentration of ribavirin (30 mg l^{-1}) gave the lowest elongation (1.9 cm) in Burren and (1.5 cm) in Binella. (Table 3).

The results showed that the treatment with 15 mA for 10 min and combined with meristem 100 μm in length gave the highest number of PVY virus-free plantlets (93% in Binella and 87% in Burren), but The rate of virus-free plantlets decreased to 62% in Binella and 57% in Burren when the treatment was 15 mA for 5 min combined with meristem (300 μm) (Table 4 and Table 5). Present results agreed with the findings of (Mahmoud et al. 2009) showing that high electric current (15 mA) for (10 min) has more affectivity for virus elimination under *in vitro* condition. Efficiency of 60 to 100% were reported for electrotherapy in the production of virus-

Table 3. Effect of Chemotherapy on the survival, growth and % of infected plants of *in vitro* plantlets of Binella and Burren.

Treatment	Meristem's length (μm)	Meristem survival rate %		Mean shoot length (cm) after 60 days		Infected plants %	
		Binella	Burren	Binella	Burren	Binella	Burren
Ribavirin 10 mg l^{-1}	100	87^{e}	88^{e}	4.33^{g}	4.85^{g}	19^{b}	25^{c}
	200	94^{c}	100^{a}	5.50^{defg}	7.30^{bc}	25^{c}	31^{d}
	300	100^{a}	100^{a}	$6.50b^{cd}$	7.80^{b}	44^{f}	50^{g}
Ribavirin 20 mg l^{-1}	100	93^{c}	88^{e}	2.10^{h}	2.33^{h}	13^{a}	19^{b}
	200	96^{b}	94^{c}	5.07^{efg}	5.57^{defg}	19^{b}	25^{c}
	300	96^{b}	94^{c}	6.43^{bcde}	7.67^{bc}	25^{c}	30^{d}
Ribavirin 30 mg l^{-1}	100	75^{h}	82^{g}	1.50^{h}	1.90^{h}	13^{a}	19^{b}
	200	91^{d}	88^{e}	4.23^{g}	4.93^{fg}	18^{b}	25^{c}
	300	97^{b}	93^{c}	6.30^{cdef}	7.40^{bc}	25^{c}	30^{d}
Control	100	86^{f}	88^{e}	4.90^{fg}	5.40^{defg}	44^{f}	31^{d}
	200	94^{c}	100^{a}	6.60^{bcd}	7.70^{bc}	38^{e}	56^{h}
	300	100^{a}	100^{a}	9.60^{a}	9.90^{a}	75^{i}	81^{j}
LSD=		1.59		1.41		2.044	

Different Ribavirin concentrations (10–20–30 mg l^{-1}) were added to the meristem culture media. The apical meristem tips in different length (100–200–300 μm) from two cultivars were excised in sterile conditions and transferred to glass tubes with 12.5 ml of meristem medium with Ribavirin. Responses with the same letter are not significantly different at $p=0.01$ by ANOVA test.

free plantlets from potato plants with a single infection of PVX (Lozoya et al. 1996). The efficiency of therapy was reported to be three to five times higher for electrotherapy in comparison with meristem culture (Lozoya et al. 1996; Meybody et al. 2011).

The success of electrotherapy in producing virus free plants depends upon both plant regeneration and virus elimination rates. The shoot elongation mean was improved by exposing shoots from Binella and Burren cultivars to electrotherapy treatment (Table 4). It was demonstrated that regeneration of Potato plant tissues could be improved by exposing explants to mild electric currents of 5 to 10 mA for 5 to 10 min (Lozoya et al. 1996).

Previous studies have shown that exposing plant tissues to electricity increases the temperature inside the cells. Denaturation of the protein moiety of virus particles may occur by increasing the temperature of the cells (Conzalez et al. 2006). It was noticed during the treatment that the temperature of potato stems was raised about (4 to 10°C), this temperature may suppress the viral activity but not inhibit the plant metabolism according to (Lozoya et al. 1996). Therefore, it has been suggested that denaturation of viral particles may occur during transport through the plasmodesmata in the apoplastic space. Inactivation of specific nucleoprotein structures leads to blockage, which prevents further penetration of virus particles to healthy cells (Gonzalez et al. 2006; Bayati et al. 2011). Also it was suggested that the stem sap pH may be changed after electric shock treatment, which may has a role in the inhibition of virus replication (Goldsworthy 1987; Retivin and Opritov 1992; Helliot et al. 2007).

The highest elongation was noticed after the treatment with 15 mA for 10 min when we used the size (300 μm) (10.6 cm) in Burren and (10.2 cm) in Binella, while the lowest elongation achieved after the treatment with 15 mA for 5 min combined with meristem size (100 μm) (5.50 cm) in Burren and (5.10 cm) in Binella (Table 4).

In conclusion, the different methods of therapies investigated were improved considerably the elimination of PVY from *in vitro* grown shoots of potato combined

Table 4. Effect of electrotherapy on the survival, growth and % of infected plants of *in vitro* plantlets of Binella and Burren.

Treatment	Meristem's length (μm)	Meristem survival rate %		Mean shoot length (cm) after 60 days		Infected plants %	
		Binella	Burren	Binella	Burren	Binella	Burren
Electrotherapy 15 mA/5 min	100	86^{d}	82^{e}	5.10^{f}	5.50^{def}	18^{c}	23^{d}
	200	88^{c}	88^{c}	6.20^{cdef}	6.90^{bcd}	25^{d}	29^{e}
	300	88^{c}	94^{b}	10.10^{a}	10.43^{a}	38^{f}	43^{g}
Electrotherapy 15 mA/10 min	100	75^{f}	75^{f}	5.50^{def}	5.80^{def}	7^{a}	13^{b}
	200	86^{d}	88^{c}	7.30^{bc}	7.45^{bc}	13^{b}	17^{c}
	300	94^{b}	100^{a}	10.20^{a}	10.60^{a}	19^{c}	24^{d}
Control	100	86^{d}	88^{c}	4.90^{f}	5.40^{ef}	44^{g}	31^{e}
	200	94^{b}	100^{a}	6.60^{bcde}	7.70^{b}	38^{f}	56^{h}
	300	100^{a}	100^{a}	9.60^{a}	9.90^{a}	75^{i}	81^{j}
LSD=		1.59		1.41		2.044	

In vitro virus-infected plantlets fixed in the electrophoresis chamber containing NaCl solution (1 N), then the electric current was applied, the amount of current and time duration applied were: 15 miliampers (mA) for 5 and 10 min. After the treatment, the apical meristem tips were excised in different lengths (100–200–300 μm) under sterile conditions and transferred to glass tubes with 12.5 ml of meristem medium.Responses with the same letter are not significantly different at $p=0.01$ by ANOVA test.

Table 5. Variations of infection rates with the virus PVY according to meristem lengths and treatments.

Meristem length	100 μm		200 μm		300 μm	
Cultivars	Burren	Binella	Burren	Binella	Burren	Binella
Treatment						
Control	44.00^{bi}	31.00^{ag}	56.00^{bk}	38.00^{ah}	81.00^{bm}	75.00^{al}
Ribavirin 10 mg l^{-1}	25.00^{bf}	19.00^{ae}	31.00^{bg}	25.00^{af}	50.00^{bj}	44.00^{ai}
Ribavirin 20 mg l^{-1}	19.00^{be}	13.00^{ad}	25.00^{bf}	19.00^{ae}	30.00^{bg}	25.00^{af}
Thermotherapy	25.00^{bf}	19.00^{ae}	31.00^{bg}	25.00^{af}	44.00^{bi}	31.00^{ag}
Electrotherapy 15 mA/5 min	23.00^{bf}	18.00^{ae}	29.00^{bg}	25.00^{af}	43.00^{bi}	38.00^{ah}
Electrotherapy 15 mA/10 min	13.00^{bd}	7.00^{ac}	17.00^{be}	13.00^{ad}	24.00^{bf}	19.00^{ae}
LSD=2.044						

*(a and b) designate a significant difference between the varieties. **(c, d, e, f,…, etc.) designate a significant difference between the varieties'treatment'meristem lengths at $P=0.01$ by ANOVA test.

with meristem culture. The best treatment was the electric chock with 15 mA for 10 min and combined with meristem culture. Electrotherapy is technically simple, fast, less costly and more effective than other techniques for eliminating virus PVY in Potato. The technique developed in this study can be useful for the production of virus free Potato germplasm, or nucleus seed stock in potato plants.

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References

Annual Statistical Abstract issued by the Central Bureau of Statistics in Damascus (2009) Syrian Arab Republic, Ministry of Agriculture, Department of Agricultural Economics, Department of Statistics

Black JD, Forsyth FR, Fensom DS, Ross RB (1971) Electrical stimulation and its effect on growth and ion accumulation in tomato plants. *Can J Bot* 49: 1805–1815

Baker R (1962) Thermotherapy of planting material. *Phytopathology* 52: 1244–1255

Bayati SH, Shams Bakhsh M, Moieni A (2011) Elimination of Grapevine virus A(GVA) by Cryotherapy and Electrotherapy. *J Agric Sci Tech* 13: 443–450

Carputo DR, Aversano, Frusciante L (2005) Breeding potato for quality traits. *Acta Hortic* 684: 55–64

Clark MF, Adams Na (1977) Characterization of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant virus. *J Genet* 34: 475–483

Conzalez JE, Sanchez R, Sanchez A (2006) Biophysical analysis of electric current mediated nucleoprotein inactivation process. *Centro Agricola* 2: 42–47

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

Dawson WO (1984) Effects of animal antiviral chemicals on plant viruses. *Phytopathology* 74: 211–213

De Fazio GJ, Caner J, Vicente M (1980) Effect of virazole (ribavirin) on tomato spotted wilt virus in two systemic hosts, tomato and tobacco. *Arch Virol* 63: 305–309

Dhital SP, Lim HT, Sharma BP (2008) Electrotherapy and chemotherapy for eliminating double-infected potato virus (PLRV and PVY) from *in vitro* plantlets of potato (*Solanum tuberosum* L.). Hortic. *Environ Biotechnol* 49: 52–57

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

Faccioli G (2001) Control of Potato Viruses using Meristem and Stem-cutting Cultures, Thermotheraphy and Chemotherapy. In: Loebenstein, G.Virus and Virus like Diseases of Potatoes and Production of Seed- Potatoes. 365–390.

Gianni F (2002) Eradication of Potato virus Y and Potato leafroll virus by chemotherapy of infected potato stem cuttings. *Phytopathol Mediterr* 41: 76–78

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

Gudmestad NC, Wambugu FM, Secor GA (1985) Eradication of potato virus Y and S from potato by chemotherapy of cultured auxiliary bud tips. *Am Potato J* 62: 667–672

Helliot B, Panis B, Hernandez R, Swennen R, Lepoivre P, Frison E (2007) Development of *in vitro* techniques for the elimination of cucumber mosaic virus from banana (Musa spp.). *Eur J Histochem* 51: 153–158

Hidayet B, Erkol D (2004) Obtaining PVX, PVY and PLRV-free micro tuber from Granola, Pasinler 92 and Caspar potato (*Solanum tuberosum* L.) cultivars. *Pak J Biol Sci* 7: 1135–1139

Hobbs HA, Reddy DVR, Rajeswari R, Reddy AS (1987) Use of Direct antigen coating and protein A coating ELISA procedure for the detection of three viruses. *Plant Dis* 71: 747–749

International Potato Center (CIP) (1993) Basic techniques in plant virology. U. Jayasinghe y L.F. Salazar (eds.). CIP, Lima (Technical Training Unit 1)

Jayasree T, Pavan U, Ramesh M, Rao AV, Reddy JM, Sadanandam A (2001) Somatic embryogenesis from leaf cultures of potato. *Plant Cell Tissue Organ Cult* 64: 13–17

Kassanis B (1957) The use of tissue culture to produce virus-free clones from infected potato varieties. *Ann Appl Biol* 45: 422–427

Khurana SMP (1992) Potato viruses & viral diseases. Tech Bull 35: 23 pp *CPRI Shimla*

Long RD, Cassells AC(1984) Elimination of virus from tissue cultures in the presence of antiviral chemicals, in plant tissue culture and its agricultural applications., *Butterworths UK*: 239–248

Lozoya S, Abello FJ (1996) Electrotherapy shoot tip culture eliminate potato virus X in potatoes. *Am Potato J* 73: 149–154

Luciana N, Gilvan PR, Lilia W, Genira PA (1993) Stock indexing and Potato virus Y elimination from potato plants cultivated *in vitro*. *Scientia Agricola* 60: 525–530

Mahmoud YM, Hosseny MH, Abdel-Ghaf MH (2009) Evaluation of some therapies to Eliminate Potato Y potyvirus from Potato plants. *Int J Virol* 5: 64–76

Meylbodi DE, Mozafari J, Babaeiyan N, Rahimian H (2011) Application of electrotherapy for the elimination of potato. *Potyvirus J Agric Sci Tech* 13: 921–927

Morel G, Martin C (1952) Guerison de dahlias atteints d,une maladie a virus *C R Acad Sci Paris*. 235: 1324–1325

Ahmad NI, Bushra T, Zakia L, Aslam JM, Saleem HM, Arshad JM, Husnain T (2010) Strategies to control potato virus Y under *in vitro* conditions. *Pak J Phytopathol* 22: 63–70

Quack F (1977) Meristem culture and virus free plants. In: J. Renert, and Y. P. S. Bajaj (Eds.). Applied and Fundamental aspects of plant cell, tissue and organ culture. Springer-Verlag New York USA, pp. 610–615

Retivin VG, Opritov VA (1992) Estimation of cold resistance of higher plants from electrophysiological analysis of their excitability. *Sov Plant Physiol* 39: 821–825

Sidwell RW, Huffman JH, Khare GP, Allen LB, Witkowski JT, Robins RK (1972) Broad spectrum antiviral activity of virazole 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxiamide. 177: 705–706

Singh RP, Valkonen JPT, Gray SM, Boonham N, Jones RA, Kerlan C, Schubert J (2008) Discussion paper: The naming of Potato virus Y strains infecting potato. *Arch Virol* 153: 1–13

Villamayor FG (1984) Growth and Yield of Potatoes (*Solanum tuberosum*) in the Lowland of the Philippines. PhD Thesis. University of Guelph.

Vinayak H, Garg ID, Sarkar D, Naik PS (1999) Amenability of Indian potato cultivars to virus elimination through meristem

culture. *Indian Potato Assoc* 26: 93–98

Yi JY, Seo HW, Choi YM, Park YE (2003) Ribavirin, electric current and shoot-tip culture to eliminate several potato viruses. *J Plant Biotechnol* 5: 101–105

Wang PJ, Hu NY (1980) Regeneration of virus-free plants through *in vitro* culture. In: A. Fiechter (Ed.), *Advances in Biochemical Engineering: plant cell culture, II* Springer-Verlag Berlin, pp 61–99