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Eradication of PVX, PVY, PVS, PVM and PLRV from Potato by Chemotherapy, Thermotherapy and Their Combinations

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Abstract:The study was carried out at Texas A&M University Potato Breeding and Variety Program in 2015, and the same study was repeated for the second time in 2016 for the reliability of the results. The five potato viruses chosen were PVX, PVY, PVS, PVM and PLRV because of their ease of inoculation by mechanical transmission. Five virus positive potato selections, virus that were obtained from the Texas A&M University Potato Breeding and Variety Program and Colorado State University Program, were used in this study. Control (tissue culture alone), chemotherapy, thermotherapy and combinations of chemotherapy+thermotherapy were used to eradicate the viruses. The control (tissue culture alone) application did not give successful results in virus eradication. Chemotherapy showed the greatest success in clearing PVM (75%-66.667%) in both years. Thermotherapy application showed the highest success in eradication of PLRV with 58.30% in the first year and 75.00% in the second year. Combining thermotherapy and chemotherapy was effective in eliminating both PVY and PLRV.

Keywords: *Solanum tuberosum*, virus eradication, chemotherapy, thermotherapy

PVX, PVY, PVS, PVM ve PLRV'nin Patatesten Kemoterapi, Termoterapi ve Kemoterapi+Termoterapi ile Temizlenmesi

Öz: Çalışma 2015 yılında Texas A&M Üniversitesi Patates Islahı ve Çeşit Programında gerçekleştirilmiş olup, sonuçların güvenirliği için 2016 yılında aynı çalışma ikinci kez tekrarlanmıştır. Seçilen beş patates virüsü, mekanik aktarım yoluyla aşılama kolaylığı nedeniyle PVX, PVY, PVS, PVM ve PLRV idi. Bu çalışmada Texas A&M Üniversitesi Patates Yetiştirme ve Çeşit Programı ile Colorado Eyalet Üniversitesi Programından elde edilen virüs pozitif patates genotipleri kullanılmıştır. Virüsleri temizlemede sadece doku kültürü uygulaması olan kontrol ile kemoterapi, termoterapi ve kemoterapi+termoterapi kombinasyonları kullanılmıştır. Sadece doku kültürüne tabi tutulan kontrol uygulaması virüs temizleme de başarılı sonuç vermemiştir. Kemoterapi uygulaması en fazla başarıyı her iki yılda da PVM'nin (75%-66.667%) temizlenmesinde göstermiştir. Termoterapi uygulaması ise ilk yıl 58.30% ikinci yıl 75.00% olmak üzere en fazla başarıyı PLRV'nin temizlenmesinde göstermiştir. Termoterapi ve kemoterapi yöntemlerinin kombine edilerek birlikte uygulanması hem PVY hem de PLRV'yi ortadan kaldırmada oldukça etkili olmuştur.

Anahtar Kelimeler: *Solanum tuberosum*, virus temizleme, kemoterapi, termoterapi

1. Introduction

Potatoes ranked third among the food crops after, wheat and rice. It originated on the high plateau of the Andes Mountains of South America and has been cultivated for some 8000 years. Recent research reports that there are 108 wild and four cultivated species of potato (Spooner et al. 2014). *Solanum tuberosum* is the most common tuber bearing species and is grown worldwide (Rowe 1993).

For potato tuber yield, the quality of seed tuber is extremely important. Fungus, bacteria, and, particularly, viruses are easily transmitted through the tubers since potatoes are vegetatively propagated (Nascimento et. al. 2003). Viral diseases lead to a decrease in vitality,

productivity, and resistance to diseases (Sangar et al. 1988).

Control of potato viral diseases are more difficult due to the inability to spray treats the plants, as growers can do with other disease-causing microorganisms (bacteria and fungi). Preventive measures are the best approach for viruses. Due to viral infection of potato tubers the ability to produce healthy plantlets from infected tubers from new varieties is essential.

Potatoes are affected by many viruses. Nearly thirty-seven virus species infect the cultivated potatoes (Beemster and de Bokx, 1987; Salazar 1996; Jeffries, 1998; Loebenstein et. al., 2001; Hull, 2002). Some of these viruses are Potato X (PVX), Y (PVY), S (PVS), M (PVM), leaf-roll (PLRV), and (POT-LV), other viruses are effective in some geographical areas (Zapata et. al. 1995; Hsu et.al. 2000; Hull 2002).

Plant viruses can be passed on through tissue cuttings and thus persist through clonally propagated material (Quak 1987; Hansen 1985). Chemotherapy (treatment with chemicals) and thermotherapy (treatment with heat) have been utilized to eliminate plant viruses (Cassells and Long, 1982; Cassells et. al. 1983; Walkey 1980). As one of the most promising antiviral drug Ribavirin, which has been synthesized in 1972 (Yang et al. 2013; Singh 2015). Thorough investigation of virus removal from infected plants has been studied in field conditions or glasshouses, and few chemicals, by itself, has been effective in eliminating or highly decreases virus titer (Tomlimson 1982; Mancino et. al., 1984; Lee et al. 1987; Lawson et al. 1990). The effectiveness of viruses' eradication depends on some conditions such as host plant, virus combination and type of virus (Knapp et al. 1995).

Thermotherapy and chemotherapy are the very feasible techniques for acquiring virus-free stocks from propagative material obtained from infected plants (Mellor and Stace-Smith 1970). The corresponding methods provide rapid proliferation of plant material and producing in good physical condition plants from a single individual in a short time. Chemotherapy has been utilized for inhibiting viruses, one that's been traditionally investigated are antimetabolites, which interfere with virus nucleic acid synthesis (Nascimento et. al. 2003). These compounds could be both synthetic and natural and they have antiviral effect. However, none show selective effect in the specific prophylaxis and large-scale treatment of plant viral diseases (Hansen and Lane 1985).

Main factors affecting plantlet survival are potato cultivar, type of virus, and the duration of heat treatment in thermotherapy method (Waswa et al. 2017). It can be suggested that further research can be carried out to improve to define suitable duration for effective getting PVX-free potato plants and this technique can be developed by working in combination with other virus elimination therapies (Cordeiro 2003; Waswa et al. 2017).

PVX eradiation was achieved by thermotherapy combined with an axillary bud of in vitro plantlets (Lozoya-Saldana and Dawson 1984; MacDonald 1973; Stace-Smith and Mellor 1968). Chemicals adding to plant tissue culture medium and shoot tips resulted in eradicating three of the major potato viruses, PVX, PVS and PVY (Klein and Livingston 1982; Wambugu et al. 1985).

Among virus species mentioned above, PLRV, PVA, PVM, PVS, PVX, and PVY have been reported to significantly affect cultivated potato and reduce potato crop production in general (Wang et al. 2011).

Clean, virus-free mother plants are required in order to produce certified seed potatoes. Unless the cultivar is resistant to a specific virus, it is necessary for the producer to acquire seed that originated from virus-free tubers. Such tubers are produced by tissue culture labs that utilize several virus eradication techniques, e.g., chemotherapy and thermotherapy. The research reported here in examines the effectiveness of these techniques individually and in combination to eradicate potato viruses including X (PVX), Y (PVY), S (PVS), M (PVM) and leaf roll virus (PLRV).

The aim of the present study is to investigate the combination of the effects of ribavirin in culture media and heat treatment for the eradication of PVX, PVY, PVS, PVM, and

PLRV from infected shoot-tips (3-4 mm) cultures.

2. Materials and Methods

The study was carried out at Texas A&M University Potato Breeding and Variety Program in 2015. The study was repeated in 2016 for the reliability of the results.

Plant Material: Five virus positive potato selections, virus that were obtained from the Texas A&M University Potato Breeding and Variety Program and Colorado State University Program, were used in this study. These selections were: TX094065 (PVY), COTX08121 (PVS, PVY; The plant had both viruses, it was a virus complex), and NDTX3248 (PLRV). The varietal names for plants with PVX and PVM were dropped over time because they were used only for positive checks in the Colorado program. Media were autoclaved at 120ºC, 10 psi for 20 minutes.

Viruses: The five potato viruses chosen were PVX, PVY, PVS, PVM and PLRV because of their ease of inoculation by mechanical transmission. The presence or absence of the virus in plants regenerated from nodes was assayed using direct antigen coating ELISA test kit reagent sets PVY, PVX, PVS, PVM and PLRV from Agdia Inc., Elkhart, IN.

Experimental Treatments: This experimental set up with five jars per variety per treatment in both year (5 jars x 4 single node cuttings x 5 viruses x 4 treatment).

Controls: Varieties were grown on standard media for a 16-hour photoperiod at 22°C for seven weeks during the same time periods of the other treatments. The selections were multiplied as nodal cuttings in jars (width 45 mm length to 90 mm) containing a standard media consisted of Linsmaier and Skoog (LS) medium (Casion Labs) (34.73 g/L) and agar (8 g/L).

Chemotherapy: Single node cuttings, 4 per jar, infected with PVY, PVX, PVS, PVM and PLRV were grown in a medium added separately with antiviral Ribavirin, at concentrations of 30mg/L. Chemotherapy treated medium was the standard media supplemented with antiviral Ribavirin (Sigma RBV) at concentrations of 30 mg/L for the different treatments. These were be placed under 16-hour photoperiod at 22°C for 7weeks, with three jars per variety per treatment. (3x6xTreatment)

Thermotherapy: Plant materials on standard media were transferred to a heat box after roots were established. Environmental conditions were at 37 ± 2ºC and 16-hour photoperiod where treatments remained for six weeks. The treated plants were removed from heat, and tips were cut and planted on standard media. After shoot growth reached 3 cm, plantlets were ELISA tested to determine if they were virus positive or virus negative.

Chemotherapy + Thermotherapy: This treatment used 30mg/L ribavirin and included moving the plant materials into the heat box for six weeks after roots were established (1-2 weeks). It is this treatment that determined the time frame of the other treatment:

Enzyme Linked Immunosorbent Assay (ELISA): ELISA test was applied for determination of virus concentration in the plants as well as identification of virus free plants (Clark and Adams 1977). The test was performed as described in the ELISA kit produced by Agdia Inc. USA. The kit contained both antibodies and enzyme conjugates. Microtiter plates with 96 wells were used to determine the virus concentration. Firstly, antibodies coated to microtiter plates for the viruses PVX, PVY, PVS, PVM and PLRV then, the plates were diluted with coating buffer (pH 9.7). Plates were incubated in a humid box overnight at 4°C. After washing the plates five times with a buffer (PBST), plant extract was mixed with buffer and placed in the wells. Plates were placed in a humid box again and incubated overnight at 4°C. The capture antibody was added to the plate with a concentration of 0.1 mg/mL alkaline phosphatase enzyme. These are diluted with ECI buffer for Virus X, Virus M, Virus S, PLRV and ECM buffer for Virus Y. After two hours incubation, plates are washed again and the PNP solution was prepared. The PNP solution was dispensed into each well (100 µl per well). Incubation was at room temperature (25ºC) for 30-60 minutes and the reaction was visually observed for the

development of yellow color. Plates were then read on a Cambridge Scientific photometer plate reader at 405 nm.

Statistical Analysis: As a result of the tests, it was determined that the data did not show normal distribution and had homogeneous variance, so arsine square root transformation was applied to the data. In addition, 0.5 was added to all data before arsine square root transformation since there were zero values in the data.

3. Results and Discussion

The effect of different treatments on five viruses is shown in Table 1. In Table 1, the ELISA test demonstrates that chemotherapy is the best treatment for PVX (%41.67-66.67), PVS (% 50.00-16.67) and PVM (% 75.00-66.67). The combined use of chemotherapy and thermotherapy was found as the most effective to eliminate PLRV (% 66.67-58.33) and PVY (%58.33 in both years) while its effect on PVM (% 33.33-50.00) elimination was also promising. The clean (virus-free) material from the first ELISA test on PVX, PVY, PVS, PVM and PLRV was tested again to verify the result. The second ELISA showed that some of the material was virus positive, the second ELISA results are included in the table above. This may be due to a small sample collected that led to a false virus-free reading.

Table 1. Effect of treatments on virus eradication (%)
Çizelge 1. *Muamelelerin virus temizlenmesi üzerine etkileri*

Treatments	Kind of Viruses									
	PVX		PVY		PVS		PVM		PLRV	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control (Tissue Culture alone)	0.00 b*	8.33 b*	0.00 b*	0.00 b*	0.00 b*	0.00 b*	0.80 c*	0.00 c*	0.00 b*	0.00 d*
Chemotherapy	41.67 a	66.67 a	0.00 b	0.00 b	50.00 a	16.67 ab	75.00 a	66.67 a	0.00 b	25.00 c
Thermotherapy	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.80 c	0.00 c	58.30 a	75.00 a
Chemotherapy +Thermotherapy	0.00 b	16.67 b	58.33a	58.33 a	8.33 b	25.00 a	33.33 b	50.00 b	66.67 a	58.33 b

* The means indicated with the same letters in the same column are not significantly different (P<0,05).

The results for the treatments so far show that there is not a set treatment that is effective for all five-virus positive plant material. This may be due to the plant varieties responding to the treatments differently or the viruses. These virus elimination results were higher than those of the Dunbar et al. (1993) who eliminated the *Peanut mottle virus* from 36%, 50% and 24% of peanut plants (Nacimento et al. 2003).

Lozoya-Saldana and Dawson (1982) reported that 24% of PVS was eradicated from shoot tip at heat treatment. The effectiveness of Ribavirin in the eradication of PVX, PVY, PVS, PVM and PLRV is reported rely on the concentration, host plant and type of infected tissue (Simpkins et al. 1981; Vicente and De Fazio 1987; Chen and Sherwood 1991; Lizarraga et al. 1991; Fletcher et al. 1998) and depends on the utilized concentration, host plant and type of infected tissue.

Studies have reported that as the concentrations of ribovirin and chemical agent increase, the success in clearing the PVA, PVM, PVS, PVX and PVY viruses in vitro plants increases. However, the increase in ribovirin concentration may cause a decrease in the viability of in vitro plants (Yang et al. 2013; Hu et al. 2015; Chahardehi et al. 2016).

Virus elimination indices support the thermotherapy method by adding antiviral agents to the potato growth medium (Fletcher et al. 1998; Dodds et al. 1989). Griffiths et al. (1990) observed the similar results, when antiviral ribavirin was added to the plant medium, virus concentration was reduced in thermotherapy. Destruction of plant viruses using an antiviral chemical such as Ribavirin is an important method for manufacture of virus-free plantlets (Khurana 2004). This technique can be simply combined with the tissue culture method to eradicate the potato viruses PVX, PVY, PVM, PLRV (Cassells and Long 1982).

4. Conclusion

In the first year of this study, PVY, PVM and PLRV elimination were observed at 58.33%,

33.33%, 66.67% of the plants by the treatment of thermotherapy + chemotherapy first year. Also, in the second year of the trial, the combined use of chemotherapy and thermotherapy was determined to be most effective for eliminating PLRV (58.33%), PVY (58.33%), and PVM (50.00%) elimination. The use of thermotherapy without antiviral therapy resulted in 58.30% in the first year and 75% in the second year PLRV-free. It was determined that chemotherapy application was most effective on PVM elimination in both years. It is also seen that this method gives successful results on PVX and PVS elimination. The control application (tissue culture alone) partially eliminated PVX, but had no effect on other viruses.

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