academicJournals

Vol. 11(3), pp. 61-70, March 2017
DOI: 10.5897/AJPS2016.1497
Article Number: 46A34D763326
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Effect of thermotherapy duration, virus type and cultivar interactions on elimination of potato viruses X and S in infected seed stocks

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Received 7 November, 2016; Accepted 28 December, 2016

Virus infection in potato reduces yield through seed degeneration. This can be reduced by use of virus-free seed tubers. However, novel approaches are required to ensure availability of virus-free stocks, especially in developing countries where the seed potato system are evolving. Consequently, a laboratory experiment was conducted at Kachwekano ZARDI in Uganda to evaluate and determine appropriate procedures for cleaning valuable potato cultivars infected with the most prevalent potato viruses; potato virus X (PVX) and potato virus S (PVS) which are frequent in the potato farming system. Thus, 20 *in-vitro* potato plantlets from cultivars Victoria, Kinigi and Rwangume each batch infected with either PVX or PVS in three replicates were grown in a thermotherapy chamber for two, three and four weeks at 37 to 40°C in 16 h of light and 30 to 34°C for 8 h of darkness per day. An equal number plantlets and replicates of the same cultivars and virus infection combinations were grown in a standard tissue culture (TC) growth room at 16 to 18°C with 16 h of light without thermotherapy as controls. Results indicated that plantlet survival after *in-vitro* thermotherapy decreased with increasing duration of heat treatment. Virus elimination efficiency significantly ($P \leq 0.05$) differed between heat-treated plantlets and the controls but not between the duration of thermotherapy treatment. However, the highest proportion of virus-free plantlets was obtained after three weeks of thermotherapy. Significant ($P \leq 0.05$) interactions were observed between thermotherapy duration and virus type on virus elimination efficiency where significantly ($P \leq 0.05$) more PVS-free than PVX-free plantlets were obtained at the same thermotherapy duration. Three weeks of thermotherapy of virus-infected *in-vitro* plantlets, particularly for PVS, offered an equilibrium duration for adequate plantlet survival and maximum meri-clone regeneration to obtain the highest proportion of virus-free plantlets. The use of thermotherapy to obtain a high proportion of PVX-free plantlets from infected potato seed stock showed no clear trend and needs further investigation.

Key words: Latent virus infection, meristem tip culture, plant virus heat therapy, recalcitrant viruses.

INTRODUCTION

Virus infection in crops is known to reduce yield and quality in global production (Salazar, 1996). Viral infections are especially problematic in vegetatively propagated crops in which they are transmitted through successive generations of infected planting material (Agrios, 2005). Therefore, production and maintenance of

virus-free planting stock of vegetatively propagated crops is crucial for controlling the spread of viruses, slowing seed degeneration and maintaining variety yield potential (Muthoni et al., 2013; Wagoire et al., 2005). Potato virus X (PVX) and Potato virus S (PVS) are the major viruses infecting potato that occur at high frequencies in most areas and potato cultivars in Uganda (Kakuhenzire et al., 2000). The two viruses are easily transmitted mechanically requiring regular monitoring and removal in infected seed if the yield potential of the cultivars is to be maintained. Potato virus X belongs to the *Potexvirus* genus and can potentially reduce yield in potato up to 10% when 100% of tubers are infected (Struik and Wiersema, 1999). This virus is inactivated at 68 to 76°C (De Bokx, 1972). Potato virus S belongs to the genus *Carlavirus,* occurs globally and may cause 10% yield loss in potato crops where 100% of seed tubers are infected (Struik and Wiersema, 1999). The thermal inactivation temperature of PVS ranges from 55 to 60°C (De Bokx, 1972). In multiple viral infections however, yield loss is enhanced that having seed free from the two viral infections becomes imperative. The common occurrence of PVS and PVX in Uganda may be attributed to the ease of virus transmission (De Bokx, 1972; Salazaar, 1996), the repeated use of the same seed stock for seed bulking with minimal renewal over time among most smallholder farmers (Tindimubona et al., 2000), the limited seed certification and finally to the lack of affordable and effective locally adapted techniques for virus detection and elimination in infected stocks. This leads to virus accumulation in potato in locally produced planting materials in potato farming systems (Kaguongo et al., 2008). Therefore, there is a need to develop and adapt appropriate techniques for cleaning and returning to the seed system existing and valuable cultivars that have either been abandoned or are being widely grown, but have very low yields due to viral infections. Recovery and restoration of valuable virus-infected cultivars can be achieved through virus cleaning or elimination in infected cultivars and maintaining them in pathogen free and reinfection-proof environment such as *in vitro* tissue culture.

Virus elimination methods can be cultivar and virus sensitive (Panattoni et al., 2013). Several methods of virus elimination have been used in potato to clean viral-infected stocks (Mahmoud et al., 2009; Dhital et al., 2008; Wang et al., 2006). Thermotherapy combined with meristem tip culture has been commonly used in several crop species for eliminating many viruses for its apparent affordability, safety and ease of generating virus-free plants (Panattoni et al., 2013). However, for a given crop such as potato, optimizing the duration high temperature exposure is required to enhance the efficiency and effectiveness of virus elimination in infected stock where positive selection is not possible to recover clean propagules. Success in plant virus elimination by meristem culture is often influenced by size of the excised meristems, crop cultivar, plant and virus species (Loebenstein et al., 2001). This procedure, however, is difficult and alone often results in a lower virus elimination efficiency (Wang and Valkonen, 2008). *In vitro* thermotherapy followed by meristem culture markedly enhances virus elimination by allowing a few specialized cells without or carrying a minimum infective virions to be excised. Excision of a few specialized cells in the apical meristem increases the frequency of meri-clone regeneration with a high proportion of virus-free plantlets (Nascimento et al., 2003; Rukarwa et al., 2010; Wasswa et al., 2010; Wang and Valkonen, 2008). Plant thermotherapy for enhancing virus elimination in infected stock involves exposing infected explants to 35 to 40°C for 14 to 28 days (Agrios, 2005). Longer thermotherapy exposure would be more effective in virus elimination, however, it compromises survival and regeneration of treated plantlets (Tan et al., 2010).

The aim of this study was to determine the effect of thermotherapy duration on potato plantlet survival and virus elimination efficiency in selected important potato cultivars in Uganda. The efficiency of virus elimination was evaluated based on plantlet survival, success of meri-clone regeneration and ratio of virus-free plantlets using different combinations of cultivar, virus type and thermotherapy duration in a laboratory conditions at Kachwekano ZARDI, Uganda.

MATERIALS AND METHODS

Plant and virus stocks

Virus infected stocks of potato cultivars Kinigi, Rwangume and Victoria were used. The stocks were infected either by Potato virus X (PVX) or Potato virus S (PVS). The virus inoculum was maintained in stocks of the three cultivars used in this study.

Production of virus-infected *in vitro* plantlets

Fully sprouted tubers of cvs Kinigi, Victoria and Rwangume obtained from field-grown, suspected virus-infected potato plants were indexed for PVX, PVS, PLRV, PVY, PVM and PVA using direct double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977). Single-tuber samples per variety obtained from plants singly infected with both PVX and PVS were planted in plastic pots in a greenhouse for viral inoculum maintenance and for obtaining viral infected ex-plants. After full germination at four leaf stage, the plants were re-indexed to ensure that only mother plants infected with either PVX or PVS per cultivar were kept and used. Thereafter, the terminal buds were excised from mother plants to promote axillary bud growth and

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Table 1. Summary of mean squares for the effect of duration of heat thermotherapy, cultivar and virus type on virus elimination efficiency in potato in Uganda in 2012

Source of variation	d.f.	Percent plantlet survival after heat treatment	Meristem survival (%) at 30 days after initiation on media	Meri-clone regeneration (%) at 45 days	Virus-free meri-clones (%)
Cultivar	2	1169.1*	73.9	4088.1*	6105.0*
Virus	1	528.1*	4644.2*	12811.7	10833*
Heat duration	3	11304.1*	276.1	5451.9*	4149*
Cultivar × Virus	2	1071.9*	840.8	1527.9	800
Cultivar × Heat duration	6	171.4*	628.2	772.7	1569
Virus × Heat duration	3	287.4*	397.8	1323.2	3665*
Cultivar × Virus × Heat duration	6	135.3*	932.2*	660.5	654
Residual	48	49.3	325.4	862.6	1067
CV (%)	-	10.5	43.2	55.0	80.5

*Represents significance levels at P≤0.05. Mean squares without asterisk were not significant at any of the levels above.

introduced in *in vitro*. The excised explants were surface-sterilized by soaking in water with liquid detergent for 30 min and then immersed in 70% ethanol for 5 s and later in a solution of 14% sodium hypochlorite and 2 drops of tween-20 for 3 min (Lizaragga et al., 1989). The disinfected explants were rinsed three times in sterile distilled water and introduced *in vitro* for plantlet regeneration. With fully developed virus-infected potato *in vitro* plantlets, single-node explants were initiated in standard tissue culture media containing 6 g agar, 100 mg ascorbic acid, 100 ml macro nutrient stock solution containing 12 ml/l $MgS0_4$, 50 ml/l calcium chloride, NH_4NO_3 (35 g/200 ml), KNO_3 (40 g/200 ml) and KH_2PO_4 (3.5 g/200 ml), 10 ml micro nutrient stock solution containing KI (0.02 g/200 ml), H_3BO_3 (0.1 g/200 ml), $MnSO_4.H_20$ (0.5 g/200 ml), $ZnS0_4.7H_20$ (0.2 g/200 ml), $Na_2MoO_4.2H_2O$ (0.005 g/200 ml), $CuSO_4.5H_2O$ (0.005 g/10 ml), $CoCl_2.6H_2O$ (0.005 g/10 ml), 5 ml $FeSO4.7H_2O$, 100 mg myoinosotol, 10 ml vitamins, , 1 ml folic acid, 4 ml L-Arginine and 30 g sucrose in 1000 ml of distilled water; pH 5.8. The regenerated plantlets were sub-cultured 3 times every 4 weeks to obtain adequate numbers of plantlets per cultivar and viral infection type for the thermotherapy experiment.

Experimental treatments

Freshly excised single-node potato stem cuttings from the virus-infected plantlets described earlier were initiated in a standard *in vitro* plant growth medium in standard tissue culture laboratory conditions (Lizaragga et al., 1989) for 2 weeks to obtain completely regenerated plantlets before exposure to thermotherapy. The fully developed, plantlets were subjected to thermotherapy at 37 to 40°C with 16 h of light (high intensity of over 10,000 lux) and at 30-34°C with eight 8 h of darkness for two, three or four weeks in a thermotherapy chamber. Twenty plantlets per cultivar, virus-infection type and thermotherapy duration treatment combinations were used. The experiment was repeated three times. The same number of virus infected plantlets per cultivar grown in standard tissue culture conditions were used as controls.

At the end of each thermotherapy period (1, 2 or 3 weeks), plantlets that had survived were recorded and apical meristem (~0.2 to 0.5 mm) were excised from each using hypodermic needles under a stereo-microscope in sterile conditions. The excised meristems were cultured on the same media for initiation in tissue culture with addition of 1.0 mg/L gibberellic acid and 0.4 mg/L benzylaminopurine to ensure better growth and regeneration of meri-clones in standard tissue culture growth room conditions (Wang et al., 2006). After one month of growth, the regenerated meristems were transferred to fresh media of the same composition for regrowth to obtain meri-clones (Wang et al., 2006). The meri-clones obtained from both heat-treated and control plantlets were sub-cultured on standard MS media devoid of growth regulators (Wang et al., 2006) to obtain fully developed plantlets that were tested to determine their virus-infection status.

Data collection and analysis

The number of plantlets per thermotherapy treatment period that survived per thermotherapy period were counted and recorded and survival percentage was computed. The regeneration efficiency of meristems was computed as a ratio of mericlones that were recoverd after meristem initiation in standard meristem tip culture medium and expressed as a percentage. Virus elimination efficiency (%) was computed as the ratio of the number of virus free meri-clones to the total number of regenerated meri-clones after full plantlet recovery and expressed as a percentage. The significance of treatment factors and their interactions on plantlet survival, meristem regeneration and virus elimination efficiency were tested using analysis of variance (ANOVA) in GENSTAT 14th Edition statistical software (VSN International, Hemel Hempstead, UK). Means of significant (P≤0.05) main effect and interactions were compared using Fishers' Protected Least Significant Difference (LSD) test at 5% probability.

RESULTS

Effect of heat treatment on potato plantlet survival and meri-clone regeneration

The survival of potato plantlets after heat treatment was significantly (P≤0.05) affected by main effects of cultivar, thermotherapy duration and type of virus infection (Table 1). First and second order interactions among the main effects significantly (P≤0.05) affected plantlet survival after heat treatment (Table 1). The success of meri-clone regeneration was significantly (P≤0.05) influenced by the cultivar and duration of heat treatment but not interaction between the two main effects (Table 1). The efficiency of

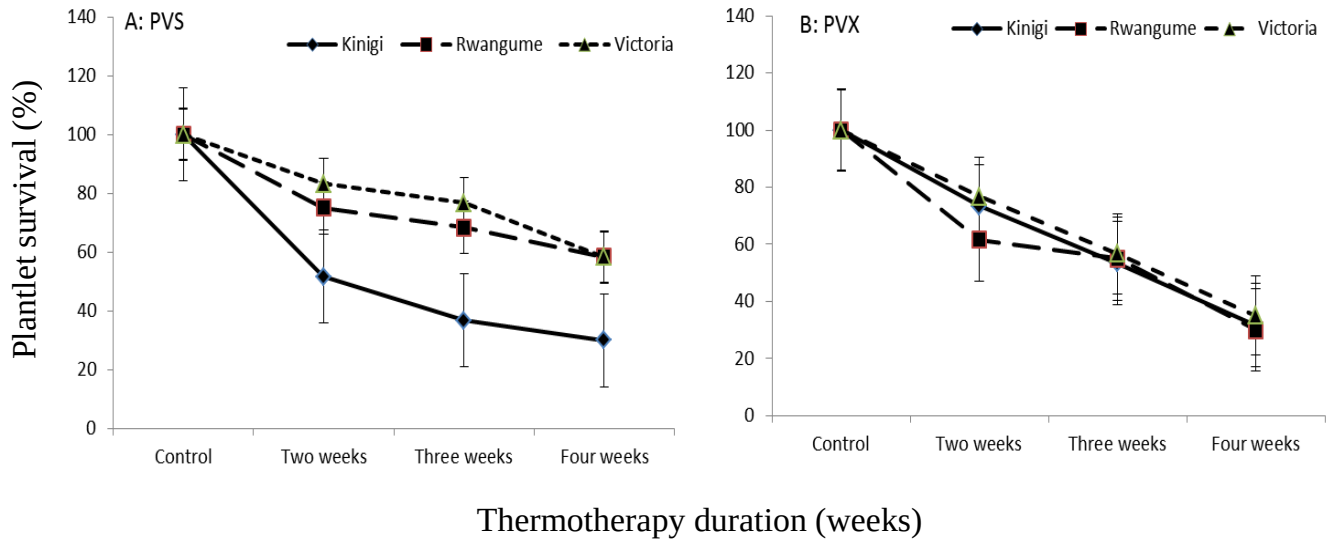

Figure 1. Percentage survival of potato plantlets infected with PVS or PVX for different duration of thermotherapy exposure.

Table 2. Survival as influenced by the two viruses.

Virus	% Survival
PVS	69.86
PVX	64.44
Mean	67.15
LSD	5.763

Table 3. Virus × cultivar interactions on potato plantlet survival after thermotherapy.

Virus	Cultivar			
	Kinigi	Rwangume	Victoria	LSD*
PVS	54.58	75.42	79.58	5.684
PVX	64.58	61.67	67.08	5.684
Mean	59.58	68.54	73.33	-
LSD	4.204	4.204	4.204	-

LSD* for Virus × Cultivar interactions.

obtaining virus-free plantlets after heat treatment was significantly (P≤0.05) influenced by virus infection type, potato cultivar, heat exposure duration and interaction between virus infection type and duration of thermotherapy (Table 1).

Effect of high temperature treatment on plantlet survival

Potato plantlet survival decreased for the three cultivars with the increase in thermotherapy duration (Figure 1) with significant difference between PVS and PVX infected stocks (Table 2). The survival % of cultivar Kinigi was significantly (P≤ 0.05) lower than that of cultivars Victoria and Rwangume which did not in turn significantly (P≤ 0.05) differ from each other when infected with PVS (Figure 1 and Table 3). There were no significant (P≤ 0.05) differences in plantlet survival after thermotherapy between cultivars from PVX-infected stocks (Table 3).

Meristem regeneration 30 days after excision and inoculation into *in-vitro* medium

The duration of thermotherapy on meristem survival at 30

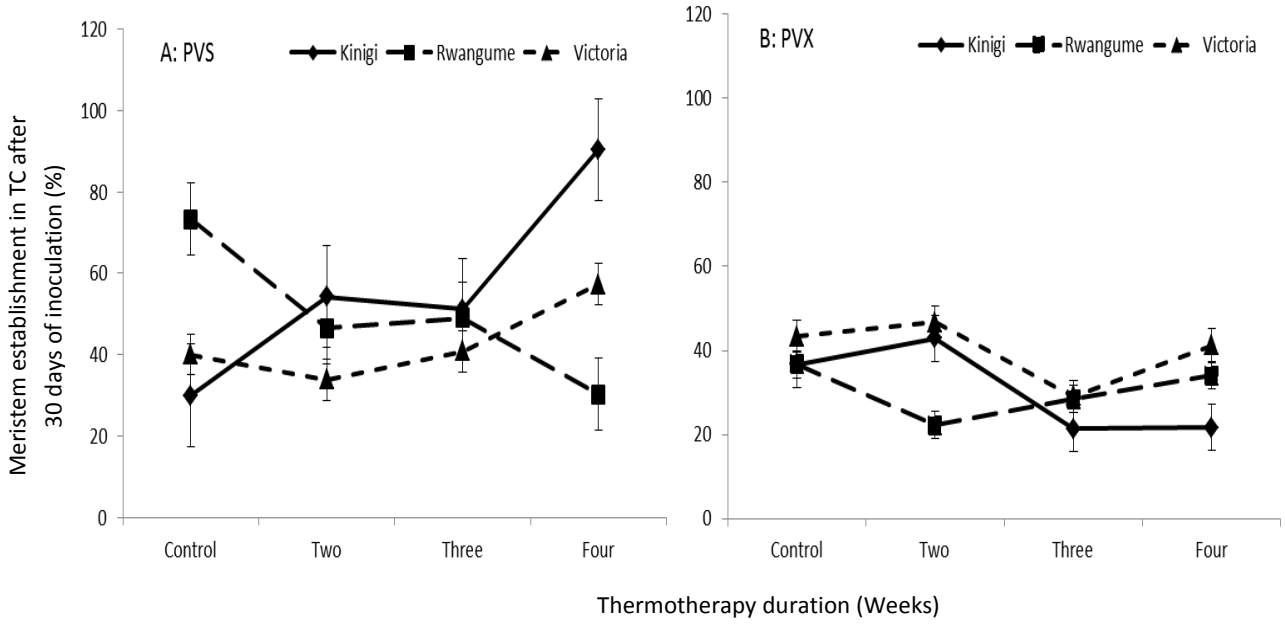

Figure 2. Effect of thermotherapy treatment duration on meristem establishment in tissue culture at 30 days after inoculation.

Table 4. Cultivar differences with respect to mericlone regeneration.

Cultivar	% Regeneration
Kinigi	54.3
Rwangume	65.3
Victoria	39.3
LSD	16.12

Table 5. Effect of thermotherapy duration on mericlone regeneration.

Thermotherapy duration (Weeks)	% Regeneration
0	27.4
2	60.7
3	66.2
4	57.5
LSD	18.62

days after excision and inoculation in meristem tip culture medium under normal tissue culture growth room conditions had no significant impact on meristem survival (Table 1). However, meristem establishment was more influenced by the type of virus present in *in vitro* plantlets (Figure 2) and affected by interaction between cultivar, virus and duration of thermotherapy (Table 1).

Effect of thermotherapy duration on potato meristem regeneration into mericlones

Cultivar Rwangume had the highest meristem regeneration efficiency to mericlones, although it did not significantly (P≤0.05) differ from cultivar Kinigi (Table 4 and Figure 3), while cultivar Victoria had the lowest meristem regeneration efficiency (Table 4 and Figure 3). Amongst the three cultivars however, the three thermotherapy durations did not significantly (P≤0.05) differ from each other, although all the heat treatment duration had higher regeneration success than the control (Table 5).

Effect of virus infection type and thermotherapy duration on virus elimination efficiency

Correlation analysis did not reveal strong relationship among measured or derived variables as factors that would influence the success of plantlet regeneration or virus elimination even when some correlation coefficients between meristem survival and virus-free mericlones was significant (P ≤ 0.05) (Table 8). Therefore, the proportion of regenerated *in-vitro* plantlets that were apparently free from viral infection after successful thermotherapy and meristem tip culture was not significantly (P ≤ 0.05) influenced by the proportion of plantlets that survived after thermotherapy and meri-clone regeneration. Cultivars Rwangume and Victoria had significantly (P≤0.05) higher rate of virus elimination than cv. Kinigi, while cv. Rwangume and cv. Victoria did not significantly (P ≤ 0.05) differ from each other (Table 6 and Figure 4). Thermotherapy exposure for two and three weeks had a significantly (P≤0.05) higher proportion of plantlets that were free from virus infection than the controls and

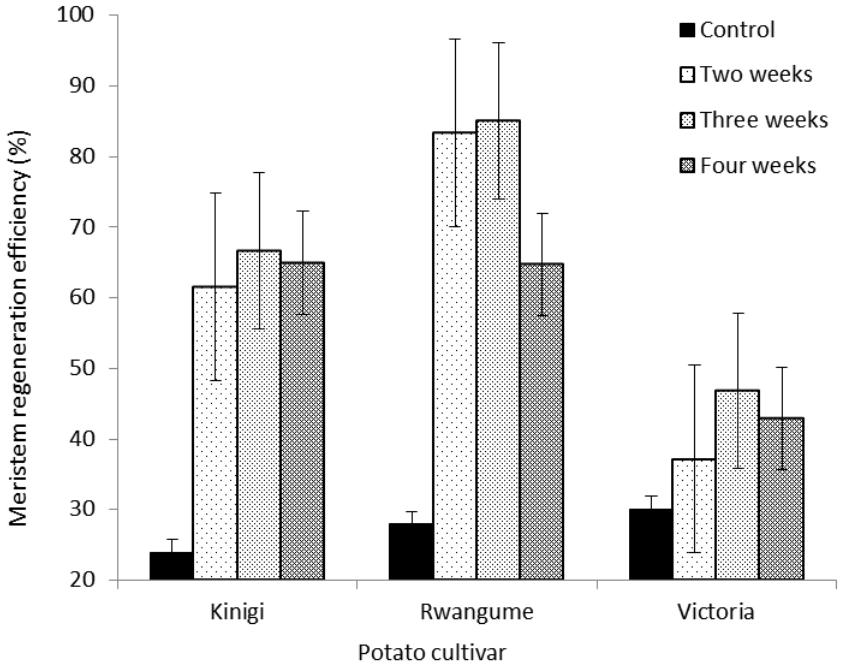

Figure 3. Effect of thermotherapy duration on potato meri-clone regeneration.

Table 6. Percent Virus elimination from each of the cultivars.

Cultivar	% Virus elimination
Kinigi	22.8
Rwangume	53.7
Victoria	45.2
LSD	19.63

plantlets that were heat-treated for four weeks (Table 7 and Figure 4).

Analysis of variance indicated significant (P≤0.05) interaction between thermotherapy duration and type of virus infection in a seed stock (Table 1). Mean comparison among main effects and interactions showed that plantlets regenerated from PVS infected seed stocks had a significantly (P≤0.05) higher virus elimination success than the ones regenerated from PVX infected mother plants (Table 9). Similarly, plantlets that were not subjected to thermotherapy had significantly (P≤0.05) lower proportion of virus-free plantlets than those that were heat treated (Table 9). However, examination of trends in success of recovering virus-free plantlets along each virus infection with increasing duration of heat treatment revealed that longer heat exposure periods increased the success of recovering virus-free plants from stock originally infected with PVS. Recovery of PVX-free plantlets after thermotherapy revealed an increasing trend in virus elimination success with thermotherapy duration up to three weeks, after which the trends in elimination decreased at the fourth week (Table 9). Nevertheless, the equilibrium of attaining a high plantlet survival after thermotherapy, high regeneration rate with high proportion of virus-free meri-clones was achieved when plantlets were exposed to heat treatment for 2 to 3 weeks (Figure 5). Thermotherapy exceeding three weeks reduced plantlet survival, meri-clone regeneration and success of recovering virus-free plantlets (Figure 5).

DISCUSSION

This study was designed to determine the effect of thermotherapy on potato plantlet survival and success of eliminating PVX and PVS infection among selected potato cultivars by varying the duration of high temperature exposure. Plantlet survival decreased with the increase in duration of thermotherapy and was significantly (P≤0.05) influenced by potato cultivar, type of viral infection and their interactions. Cultivar Victoria tolerated longer periods of thermotherapy than the other two cultivars indicating different levels of tolerance to extended high temperature exposure by different potato cultivars. However, this is likely to compromise the success of virus elimination since it reduced plantlet survival. The low survival rates for Kinigi and Rwangume even at low thermotherapy exposure periods implied that these cultivars have low tolerance to high temperature (Ali et al., 2013). The higher survival rate of cultivar Victoria after four weeks of heat treatment than the other

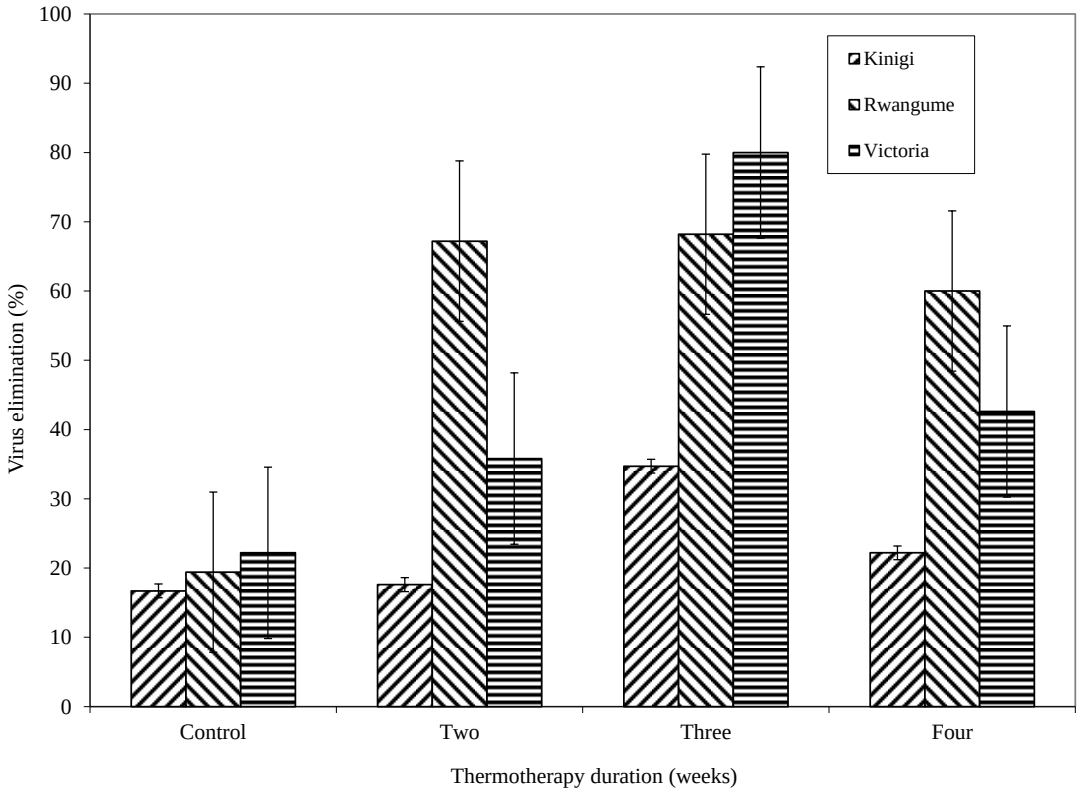

Figure 4. Effect of thermotherapy duration of virus elimination success for different potato cultivars.

Table 7. Percent Virus elimination under each of the Thermotherapy exposure durations.

Thermotherapy duration	% Virus elimination
0	19.4
2	45.9
3	55.3
4	41.6
LSD	22.67

Table 8. Correlation coefficient among plantlet survival after heat treatment (%), meristems survival (%) in tissue culture at 30 days after excision, meri-clone regeneration after 45 days (%) and virus free meri-clones (%).

Variable	Survival (%) after heat treatment	Meristems survival (%) in TC at 30 days after excision	Meri-clone regeneration after 45 days (%)	Virus free meri-clones (%)
Plantlet survival after heat treatment (%)	0			
Meristems survival (%) in TC at 30 days after excision	-0.017	0		
Meri-clone regeneration after 45 days (%)	-0.229	0.155	0	
Virus free meri-clones (%)	-0.034	0.089	0.488***	0

***Implies significant correlation.

cultivars was probably due to its ability to thrive in diverse environments in nature in contrast to cvs. Kinigi and Rwangume which are adapted to cooler agro-ecologies. Nevertheless, cultivars Rwangume and Kinigi had higher

Table 9. Effect of virus infection type and thermotherapy duration on virus elimination efficiency in infected stocks.

Type of virus	Thermotherapy duration				Mean
	0	2	3	4	
PVS	16.7	61.4	61.1	72.1	52.825
PVX	22.2	30.4	49.4	11.1	28.275
Mean	19.45	45.9	55.25	41.6	40.55
LSD	22.67	22.67	22.67	22.67	18.31

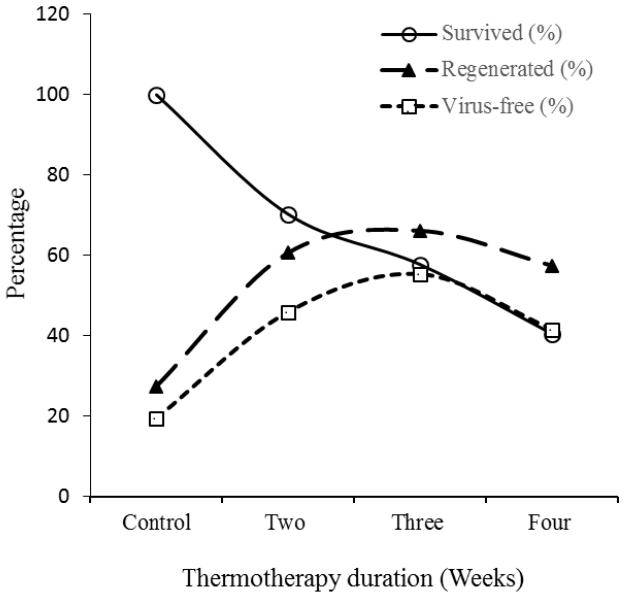

Figure 5. Equilibrium between plantlet survival after thermotherapy and mer-iclone regeneration with virus-elimination success.

meristem regeneration efficiencies implying differences in cultivars for meristem culture and meri-clone regeneration (Cheong et al., 2014; Danci et al., 2012; Biniam and Tadesse, 2008).

Virus-free meri-clones were obtained in both heat treated and controls plantlets, however, the virus-free success rate was higher in the former than the latter. The high virus elimination success in heat treated *in-vitro* plantlets may be due degradation of virions at extended high temperature exposure that resulted in unfavorable conditions for virus replication due to competition among the rapidly diving host cells and replacing virus particles (Hull, 2002). The low virus elimination efficiency of plantlets that were in thermotherapy for four weeks may be attributed to the difficulty in excising the meristems from weakened plantlets. This could have been largely contributed by picking a large portion of already differentiated cells possibly containing infectious virus particles (Biniam and Tadesse, 2008).

In this study, virus elimination efficiency was influenced by the main effects of cultivar, thermotherapy duration, virus type and interaction between thermotherapy duration and type of virus. Among the tested cultivars, Rwangume had the highest overall rate of virus elimination. Based on the data obtained in this study, the absence of significant interaction between potato cultivar and thermotherapy duration indicated that the success of virus elimination at any heat exposure duration was independent of the potato cultivar but influenced by the duration of thermotherapy and the type of virus infection involved. The success of virus elimination generally increased with the duration of thermotherapy up to the third week. The decrease of virus-free plants due to fourth week of thermotherapy could not be explained by this data considering that virus elimination success was not related to plantlet survival or meri-clone regeneration. However, interaction between heat treatment duration and virus type was significant where a high proportion of formerly PVS-positive plantlets were virus-free by the three weeks of heat treatment than stocks that were previously infected with PVX. This implies that the success of virus elimination depends on the virus species possibly due to the existence of complex interactions between the host plant cultivar and the virus species in the infected stock (Panattoni and Triolo, 2010; Panattoni et al., 2013).

The success of virus elimination was the highest for three weeks of thermotherapy especially for stocks that were previously infected with PVS, although there was no significant ($P \leq 0.05$) difference in virus elimination success among heat treatment durations except the controls. However, the impact of high temperature exposure is clearly depicted by the significant difference between heat treated plants and the controls in both PVX and PVS infected stocks. The lower success in obtaining virus-free plants from the stocks that were previously infected with PVX may be related to differences in virus inactivation temperature which is higher for PVX than PVS (Fernow et al., 1962; De Bokx, 1972; Hull, 2002). Thus, accumulated heat-units over the four week heat treatment period were probably not adequate to result in effective destruction or retardation of PVX replication to obtain more PVX-free meri-clones (Lizarraga et al., 1989).

The proportion of PVX-free plantlets after heat treatment could increase by extending the thermotherapy period. However, this may negatively affect plantlet survival, making it difficult to extract a virus-free apical dome and thus reducing the ability of meri-stems to regenerate in tissue culture. Alternatively, the low success of PVX elimination by thermotherapy and meristem tip culture could be attributed to the smaller size of PVX (470-480 nm long and 13 nm wide) than PVS (610-700 nm long and 12 to 15 nm wide) (Hull, 2002). This means that PVX can easily move through cells via the plasmodesmata up to the meristematic tip rendering PVX more recalcitrant for removal in infected potato stem

tissue than PVS (Hsu et al., 2000; Loebenstein et al., 2001).

CONCLUSION AND RECOMMENDATIONS

This study demonstrated that the success of cleaning potato stock previously infected with PVX or PVS using thermotherapy is significantly influenced by the potato cultivar, type of virus and duration of heat treatment. Data further showed that there was no significant interaction between potato cultivar and thermotherapy duration indicating that cultivars used in this study do not need adjustments to heat treatment duration in order to obtain equivalent success in virus elimination. The study additionally showed that plantlet survival decreased with increase in duration of thermotherapy and PVX-infected stock possibly requires a longer thermotherapy exposure period than PVS infected material. However, extended high temperature exposure led to reduced plantlet survival, low meri-clone regeneration and, in consequence, low virus elimination success. Virus-infected plantlets subjected to meristem tip-culture without thermotherapy also generated some virus-free meri-clones after meristem tip culture but frequencies were lower. In heat-treated virus-infected stock, the proportion of virus-free plantlets increased with longer thermotherapy periods (up to three weeks) particularly for PVS. The efficiency of virus elimination was influenced by virus type and potato cultivar on one hand and thermotherapy duration with virus type interactions on the other. However, interaction between thermotherapy and type of virus present was significant as indicated by the low success of removing PVX as compared to PVS. It is therefore imperative to conduct further studies to determine more appropriate duration of thermotherapy beyond the highest exposure period that was tested in this study for maximum success in obtaining PVX-free plants. However, this may negatively impact plantlet survival, success of meri-stem excision and meri-clone regeneration and consequent recovery of PVX-free plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank Kachwekano Zonal Agricultural Research and Development Institute (KAZARDI) of the National Agricultural Research Organization (NARO) for the providing tissue culture and serology research facilities and support staff to undertake this work. The Millennium Science Initiative through the Uganda National Council for Science and Technology for funding this project as part of M.Sc. training for the first author is specially recognized.

REFERENCES

Agrios GN (2005). Plant Pathology (5th Ed). Academic Press, Burlington, USA. 948 p.

Ali M, Nasiruddin K, Haque M, Faisal S (2013). Virus elimination in potato through meristem culture followed by thermotherapy. SAARC J. Agric. 11:71-80

Biniam T, Tadesse M (2008). A survey of viral status on potatoes grown in Eritrea and in vitro virus elimination of a local cultivar 'Tsaeda embaba'. Afr. J. Biotechnol. 7:397-403.

Cheong EJ, Jeon AR, Kang JW, Mock R, Kinard G, Li R (2014). *In vitro* elimination of Black raspberry necrosis virus from black raspberry (*Rubus occidentalis*). Hortic. Sci. 41:95-99.

Clark MF, Adams AN (1977). Characteristics of the micro-plate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 134:475-483.

Danci M, Danci O, Baciu A, Olaru D, Petolescu C, Berbentea F, David I (2012). Production of virus-free potato plantlets. J. Hortic. For. Biotechnol. 16:232-238.

De Bokx JA (1972). Viruses of potatoes and seed-potato production. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands. 233 p.

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.

Fernow KH, Peterson LC, Plaisted RL (1962). Thermotherapy of *Potato leaf roll Virus*. Am. Potato J. 39: 445-451.

Hsu YH, Annamalai P, Lin CS, Chen YY, Chang WC, Lin NS (2000). A sensitive method for detecting bamboo mosaic virus (BaMV) and establishment of BaMV-free meristem tip cultures. Plant Pathol. 49:101-107.

Hull R (2002). Matthews' Plant Virology, 4th Edition. Academic Press, Inc. New York. 1001p.

Kaguongo W, Gildemacher P, Demo P, Wagoire W, Kinyae P, Andrade, J, Forbes G, Fuglie K, Thiele G (2008). Farmer practices and adoption of improved potato cultivars in Kenya and Uganda. International Potato Center (CIP), Lima, Peru. Social Sciences Working Paper. 96p.

Kakuhenzire R, Hakiza JJ, Mateeka B, Lemaga B, Salazar L, Olanya M (2000). Incidence and importance of potato viruses in south-western Uganda. African Potato Association Conference Proceedings, 5:285-290.

Lizarraga R, Espinosa N, Jayashinghe U, Dodds JH (1989). Tissue Culture for elimination of pathogens. CIP Research Guide 3. CIP Lima.

Loebenstein G, Berger PH, Brunt AA, Lawson RH (2001). Virus and virus-like diseases of potatoes and production of seed potatoes. The Netherlands: Kluwer Academic Publishers. 460p.

Mahmoud SYM, Hosseny MH, Abdel-Ghaffar MH (2009). Evaluation of some therapies to eliminate *Potato virus Y* from potato plants. Int. J. Virol. 5:64-76.

Muthoni J, Shimelis H, Melis R (2013). Potato production in Kenya: Farming systems and production constraints. J. Agric. Sci. 5:182-197.

Nascimento LC, Pio-Ribeiro G, Willadino L, Andrade GP (2003). Stock indexing and *Potato virus Y* elimination from potato plants cultivated in vitro. Sci. Agric. 60:525-530.

Panattoni A, Triolo E (2010). Susceptibility of grapevine viruses to thermotherapy on *in vitro* collection of Kober 5BB. Sci. Hortic. 125:63-67.

Panattoni A, Luvisi A, Triolo E (2013). Review. Elimination of viruses in plants: twenty years of progress. Span. J. Agric. Res. 11:173-188.

Rukarwa RJ, Mashingaidze AB, Kyamanywa S, Mukasa SB (2010). Detection and elimination of sweetpotato viruses. Afr. Crop Sci. J. 18:223-233.

Salazar LF (1996). Potato Viruses and their Control. Lima, Peru: International Potato Center. 214p.
Struik PC, Wiersema SG (1999). Seed and Potato Technology. Purdue University Press, The Netherlands. 382 p
Tan RR, Wang LP, Hong N, Wang GP (2010). Enhanced efficiency of virus elimination following thermotherapy of shoot-tip cultures of pear. Plant Cell Tissue Organ Cult. 101:229-235
Tindimubona S, Kakuhenzire R, Hakiza JJ, Wagoire WW, Beinamaryo J (2000). Informal production and dissemination of quality seed potato in Uganda. African Potato Association Conference Proceedings 5:99-104.
Wagoire WW, Kakuhenzire R, Kashaija IN, Lemaga B, Demo P, Kimmone G (2005). Seed potato production in Uganda: Current status and future prospects. African Crop Science Conference Proceedings 7:739-743.
Wang Q, Liu Y, Xie Y, You M (2006). Cryotherapy of Potato Shoot Tips for Efficient Elimination of *Potato leafroll virus* (PLRV) and *Potato virus Y* (PVY). Potato Res. 49:119-129.
Wang QC, Valkonen JPT (2008). Efficient elimination of Sweetpotato little leaf phytoplasma from sweet potato by cryotherapy of shoot tips. Plant Pathol. 57:338-347.
Wasswa P, Alicai T, Mukasa SB (2010). Optimization of *in vitro* techniques for Cassava brown streak virus elimination from infected cassava clones. Afr. Crop Sci. J. 18:235-241.