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Research Appication Summary

Effect of cultivar and thermotherapy combined with meristem-tip culture on eliminating prevalent viruses infecting potato in Uganda

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Abstract

Potato (*Solanum tuberosum*) is an important crop in eastern and south-western highlands agro-ecological zone of Uganda where it is suitable for bridging periods of food shortage. However, production is constrained by viruses which reduce yield through accelerating tuber degeneration. Current management practices include serological indexing of mother plants to identify virus free plants for initiating in tissue culture. Healthy tissue culture plantlets are subsequently used to produce clean mini-tubers which are bulked in open fields to obtain sizeable amounts of pre-basic and basic seed. This process is characterized by low multiplication rates, requiring several generations of field multiplications to produce sizable amounts of seed. Repeated multiplications result in progressive virus infections leading to seed degeneration and subsequently dropping high yielding cultivars from the seed system. This process can be reversed by efficient virus elimination methods whose success depends on type of virus, plant species and cultivar type. Therefore this study generally aimed at evaluating and determining appropriate procedures for virus elimination from selected potato cultivars in Uganda. Specific objectives of the study were: (i) to establish the incidence and distribution of major potato viruses in key potato producing districts of Uganda and then (ii) establish the optimum temperature exposure period for maximum virus elimination from selected virus-infected potato cultivars. Leaf Samples were collected from farmers' fields and assayed for viruses using double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) aided by a plate reader at 405 nm wavelength. Prevalent viruses were eventually subjected to thermotherapy for 0, 2, 3 or 4 weeks at 37-40^{0}C; 16 hours of light and 30-34^{0}C; and 8 hours of darkness. The study identified four of the six most important viruses; Potato virus X (PVX), Potato virus S (PVS), Potato virus M (PVM) and Potato leafroll virus (PLRV) at 64.9, 78.9, 3.1 and 21.2 % incidencies respectively across districts. Results indicated that virus elimination efficiency significantly ($P \leq 0.05$) varied between the four virus elimination treatments, cultivars and viruses.

Key words. Cultivar, meristem-tip culture, solanum tuberosum thermotherapy, virus, virus elimination

Résumé

La pomme de terre (*Solanum tuberosum*) est une culture importante dans la zone agro-écologique des hautes montagnes du sud-ouest de l'Ouganda, où elle est apte à combler des périodes de pénurie alimentaire. Cependant, sa production est limitée par des virus qui réduisent le rendement en accélérant la dégénérescence des tubercules. Les pratiques actuelles de gestion incluent l'indexation sérologique des plantes mères pour identifier les plantes sans virus pour l'initiation dans la culture tissulaire. Des plantules saines issues de la culture de tissulaire sont ensuite utilisées pour produire des mini-tubercules saines qui sont entreposées dans des champs pour obtenir des quantités importantes de semences pré base et de semence de base. Ce processus est caractérisé par de faibles taux de multiplication, nécessitant plusieurs générations de multiplications pour produire des quantités importantes de semence. Les multiplications répétées entraînent des infections virales progressives conduisant à une dégénérescence des semences et, par la suite, la suppression de cultivars à haut rendement du système semencier. Ce processus peut être inversé par des méthodes efficaces d'élimination du virus dont le succès dépend du type de virus, des espèces végétales et du type de cultivar. Par conséquent, cette étude visait de façon globale à évaluer et à déterminer les procédures appropriées pour l'élimination du virus chez certains cultivars de pommes de terre en Ouganda. Les objectifs spécifiques de l'étude étaient les suivants: i) établir l'incidence et la répartition des principaux virus de la pomme de terre dans les principaux districts de production de pommes de terre en Ouganda; ii) établir la période optimale d'exposition à la température pour l'élimination maximale du virus de certains cultivars de pommes de terre infectés. Des échantillons de feuilles ont été prélevés dans les champs des agriculteurs et testés en utilisant un dosage immuno-enzymatique à double enzyme d'anticorps (DAS-ELISA) assisté par un lecteur de plaques à une longueur d'onde de 405 nm. Les virus prévalant ont finalement été soumis à une thermothérapie pendant 0, 2, 3 ou 4 semaines à 37-400 ° C; 16 heures de lumière et 30-340C; et 8 heures d'obscurité. L'étude a identifié quatre des six virus les plus importants; virus de la pomme de terre X (PVX), virus de la pomme de terre S (PVS), virus de la pomme de terre M (PVM) et virus de la feuille de pomme de terre (PLRV) avec des incidences respectives de 64,9, 78,9, 3,1 et 21,2% dans les districts. Les résultats indiquent que l'efficacité d'élimination du virus a varié de façon significative ($P \leq 0,05$) entre les quatre traitements d'élimination du virus, les cultivars et les virus.

Mots clés. Cultivar, culture du méristème, solanum tuberosum thermotherapy, virus, élimination du virus

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Background

In potato production, seed accounts for 40-50% of the variable costs rendering it the most expensive and major input in this agro-enterprise (Tindimubona *et al.,* 2000). Efforts by organizations such as National Agricultural Research Organization (NARO) and Uganda National Seed Potato Producers' Association (UNSPPA) to avail quality seed to farmers have not been very successful due to limited quantities of starter seed

and high cost of seed. Many farmers consequently opt for home-saved seed, seed from neighbors or local markets. This combined with poor cultural practices results in low potato productivity. In order to improve potato productivity with improved cultivars and those conserved by farmers, the supply of quality seed among other production variables is imperative and has to be improved. This can be achieved by indexing of seed for virus infection, removal of stocks that are infected, and advance in production of disease free materials. However, where clean stock cannot be obtained, removal of viruses in infected material is necessary to obtain initial clean stock to restore the yield potential of cultivars of interest (Kakuhenzire *et al.,* 2000). Thermotherapy combined with meristem-tip culture is the most commonly applied technique to eliminate viruses from infected plant materials of different plant species (Panattoni *et al.,* 2013). Therefore this study aimed at evaluating and determining appropriate procedures for virus elimination from selected potato cultivars in Uganda.

Literature summary

Potato (*Solanum tuberosum* L.) is one of the most extensively grown crops in the highland regions of the world (Karim *et al.,* 2010). In Uganda, potato is produced by more than 200,000 households on 100,000 hectares yielding about 680,000 tons annually (FAOSTAT, 2011). Approximately 70% of potato in Uganda is produced from Kisoro, Kabale and Kanungu districts in south-western region, and Mbale, Kapchorwa in the eastern Region (Anon, 2007). Most of the potato crop in these areas is grown from home-saved seed (Wagoire *et al.,* 2005). Such seed has undergone extensive recycling for several seasons without replenishment, resulting in progressive accumulation of degenerative diseases mainly viruses (Wagoire *et al.,* 2005). Once infected with viruses, the potato stock will remain infected for life unless regenerated through strict virus elimination protocols (Khurana, 2004). Efficient virus elimination protocols will ensure regular availability of clean seed to farmers. Techniques such as in-vitro thermotherapy combined with meristem-tip culture have been used to eliminate potato viruses such as PVX and PVS (Biniam and Tadesse, 2008). EL Far and Ashoub (2009) and Mashilo *et al.* (2012) achieved 95.3 and 100 % elimination of Sweetpotato featherly mottle virus by applying thermotherapy combined with meristem-tip culture respectively. Thermotherapy has also been successfully used in the elimination of Sweet potato mild mottle virus (SPMMV) and Sweet potato feathery mottle virus (SPFMV) (Rukarwa *et al.,* 2010), and African cassava mosaic virus (Wasswa *et al*., 2010). In Uganda, these methods have not been evaluated among cultivars of importance in order to restore their yield potential. In order for this to be effected, there is need to first understand the prevalent viruses infecting potato in Uganda.

Study description

A field survey was conducted during the first (March-June) and second (August-November) rainy season of 2012 in Kabale, Kanungu and Kisoro districts in south-western Uganda and, Kapchorwa and Mbale districts in eastern Uganda. Potato

leaf samples were collected from south-western Uganda at altitudes ranging from 1048 to 2478 meters above sea level (m. a. s. l); latitudinal range of 000 49.634' in Kanungu to 010 28' in Kabale and longitudinal range of 290 37.178' in Kanungu to 300 07.936' in Kabale. For eastern Uganda, samples were collected from altitude 1134 to 2582 m. a. s. l. The latitudinal range was 000 59' in Mbale to 010 26' in Kapchorwa and longitude 340 10.542' in Mbale to 340 35.330' in Kapchorwa. At least 40 potato fields each about half an acre (0.25 ha) were randomly selected at about 5 km intervals along main and feeder roads traversing each district. In each field, twenty leaf samples from both symptomatic and asymptomatic plants were collected along the borders and diagonals and delivered in numbered plastic bags (Kakuhenzire *et al.*, 2000). The virus load was estimated at 405 nm filter using an ELISA micro-titration plate reader. A sample was considered infected if the light absorbance value at 405 nm filter was greater than or equal to the mean of ten healthy controls plus three standard deviations in a given plate. Tubers of preferred cultivars; Kinigi, Victoria and Rwangume were planted in plastic pots and indexed for the six most important potato viruses; PVX, PVS, PLRV, PVY, PVM and PVA using DAS-ELISA protocol described by (Clark and Adams, 1977). Only plants with single virus infections of the most prevalent viruses (PVX or PVS) were retained. Auxiliary or terminal buds were excised from these plants and surface sterilized following the protocol of Zapata *et al.* (1995). The disinfected explants were rinsed three times in sterile distilled water and initiated on MS growth media. The plantlets were sub-cultured after every four weeks to obtain the required number of plantlets for the thermotherapy experiment. Thermotherapy was conducted by exposing each cultivar x virus infection combination to 37-40^0 C for 16 hours of light and 30-34^0 C for 8 hours of darkness for 0, 2, 3 or 4 weeks. After each high temperature exposure period, apical meristems (~ 0.1-0.5 mm) were excised under a stereo-microscope and cultured on basal growth media containing 1.0 mg/L gibberellic acid and 0.4 mg/L benzylaminopurine (Wang *et al.,* 2006) to obtain mericlones. Mericlones were then tested to determine virus elimination efficiency.

Data analysis

Percentages for positive samples were analyzed using GenStat 14th Edition. Significant differences among virus elimination treatments were detected using analysis of variance (ANOVA) in GENSTAT 14th Edition statistical software and significant means compared using Fishers' Protected least significance difference test at 5% probability.

Research Application

Among the six viruses surveyed, PVY and PVA were not detected in any of the five main potato producing districts. Incidence of the other four viruses; PLRV, PVX, PVS and PVM was significantly ($P<0.05$) influenced by the potato cultivar and districts where the samples were collected. Interaction between district and season significantly influenced the incidence of PVX, PVS and PVM (Table 1).

Table 1: Mean squares for effect of cultivar, cropping system, season and district on virus incidence in Uganda in 2012

Source of variation	d.f.	PLRV	PVX	PVS	PVM
Cultivar	21	2497***	2304***	1923.8*	72.02
Cropping system	1	95.5	1755.5	46.9	546.92*
Season	1	2230	529.6	1706.8	6.54
District	4	6377.3***	7033.4***	3351.6*	44.66
Cropping system x Season	1	760.2	294.2	0.7	48
Cropping system	4	586.1	1677.9	203.2	127.68
Season x District	4	609.7	5761.7***	3518.7*	191.01*
Cropping system x Season x District	4	11.1	1004.1	90.6	85.9
Residual	65	745.5	758.7	795.1	53.11

*, **, and *** represent significance levels at P<0.05, P<0.01 and P<0.001, respectively. Mean squares without asterisk were not significant at any of the levels above.

Across locations, the most prevalent virus was PVS followed by PVX, PLRV and PVM, respectively (Table 2). The incidence of Potato virus M (29.6 %) and PVS (95.3 %) were significantly higher in the eastern than south-western region while the incidence of PLRV (12.7 %) was significantlyhigher in the south-western region.

Table 2: Incidence (%) of the different potato viruses in major potato producing districts of Uganda in 2012

	South-western				Eastern			
Virus	Kabale	Kanungu	Kisoro	Mean	Kapchorwa	Mbale	Mean	Overall mean
PVS	61.7	65.6	60.4	62.6	96.9	93.8	95.3	78.9
PVX	39.5	52.6	90.0	60.7	60.0	78.0	69.0	64.9
PLRV	12.7	9.4	66.6	29.6	17.7	7.8	12.7	21.2
PVM	2.3	0.0	0.0	0.8	4.7	6.2	5.4	3.1

The success of eliminating the most prevalent viruses (PVX and PVS) was significantly (P<0.05) influenced by type of virus present in in-vitro potato plantlets, potato cultivar and heat exposure duration (Table 3). Interaction between type of virus eliminated and thermotherapy duration also significantly (P<0.05) affected virus elimination efficiency (Table 3).

Table 3: Mean squares for effect of duration of high temperature exposure, cultivar and virus type on virus elimination efficiency in potato in Uganda in 2012

Source of variation	d.f.	Plantlet survival (%) after heat treatment	Meri-clone regeneration (%)	Virus free meri-clones (%)
Replications	2	2.4	41.7	220.0
Virus	1	528.1	12811.7	10833.0*
Clone	2	1169.1***	4088.1**	6105.0**
Heat duration	3	16887.4***	5451.9***	4149.0*
Virus x Clone	2	1071.9***	1527.9	800.0
Virus x Heat duration	3	287.4**	1323.2	3665.0*
Clone x Heat duration	6	171.4**	772.7	1569.0
Virus x Clone x Heat duration	6	135.3*	660.5	654.0
Residual	44	52.2	768.2	1139.0
C.V. (%)		10.8	52.4	83.2

*, **, and *** represent significance levels at P<0.05, P<0.01 and P<0.001, respectively. Mean squares without asterisk were not significant at any of the levels above.

Potato virus S had a higher elimination rate (72.1%) than Potato virus X (11.1%) after exposing plants to a four-week thermotherapy exposure duration. The highest elimination efficiency for PVS was attained at a four-week thermotherapy exposure duration (72.1%) while meristem-tip culture had the lowest (16.7%). For PVX, the highest virus elimination rate (49.4%) was attained at three-week thermotherapy duration. Of the three cultivars, Rwangume had the highest overall rate of virus elimination (Fig. 1). Thermotherapy exposure for three weeks had the highest overall virus elimination efficiency as compared to the controls where meristems were excised without thermotherapy (Fig. 1). Although more plantlets freed of viruses were obtained at three weeks thermotherapy exposure, the three high temperature exposure durations were not significantly (P<0.05) different from each other (Fig.1)

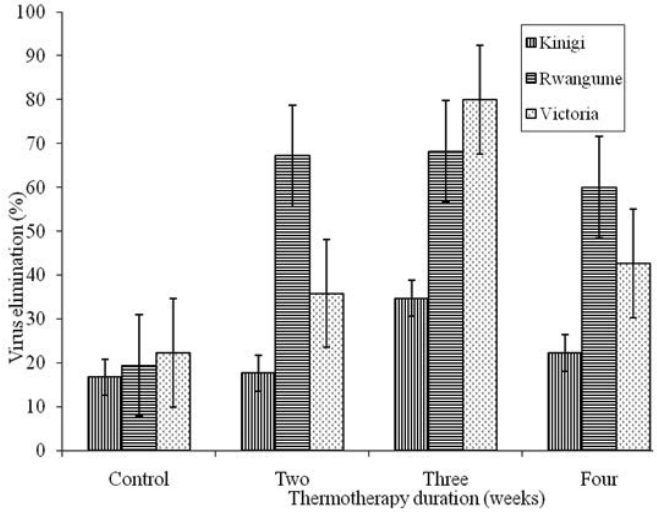

Figure 1. Overall percentage virus elimination from different potato cultivars under different thermotherapy exposure durations

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References

Anon, 2007. Fresh Irish potato Sub-sector study in Kabale District, Uganda. Contributing to poverty eradication through community empowerment and farmers' access to markets. 32pp.

Biniam, T. and Tadesse, M. 2008. A survey of viral status on potatoes grown in Eritrea and in vitro virus elimination of a local cultivar 'Tsaeda embaba'. *African Journal of Biotechnology* 7: 397-403.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the micro-plate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 134:475-483.

FAOSTAT, 2011. Food and Agriculture Organization of the United Nations. Production Statistics, Rome Italy (www.faostat.fao.org) accessed 17/01/2013.

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

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

Khurana, S. 2004. Potato viruses and their management. *Diseases of Fruits and Vegetables* 2: 389-440.

Karim, M. R., Hanif, M. M., Shahidullah, S. M., Rahman, A. H. M. A., Akanda, A. M. and Khair, A. 2010. Virus free seed potato production through sprout cutting technique under net-house. *African Journal of Biotechnology* 9: 5852–5858.

Mashilo, J., Van Niekerk, R. and Shanahan, P. 2012. Combined thermotherapy and meristem tip culture for efficient elimination of feathery mottle virus in sweet potato (*Ipomoea batatas*). *Acta Horticulturae* 1007:719-726.

Mervat, M. M. El Far. and Ashoub, A. 2009. Utility of thermotherapy and meristem tip for freeing sweetpotato from viral infection. *Australian Journal of Basic and Applied Sciences* 3 (1): 153-159.

Panattoni, A., Luvisi, A. and Triolo, E. 2013. Review. Elimination of viruses in plants: twenty years of progress. *Spanish Journal of Agricultural Research* 11: 173-188.

Rukarwa, R. J., Mashingaidze, A. B., Kyamanywa, S. and Mukasa, S. B. 2010. Detection and elimination of sweetpotato viruses. *African Crop Science Journal* 18:223-233.

Tindimubona, S., Kakuhenzire, R., Hakiza, J. J., Wagoire, W. W. and Beinamaryo, J. 2000. Informal production and dissemination of quality seed potato in Uganda. *African Potato Association Conference Proceedings* 5: 99-104.

Wagoire, W. W., Kakuhenzire, R., Kashaija, I. N., Lemaga, B., Demo, P. and 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. and You, M. 2006. Cryotherapy of potato shoot tips for efficient elimination of *Potato leafroll virus* (PLRV) and *Potato virus Y* (PVY). *Potato Research* 49: 119–129.

Zapata, C., Miller, J. C. and Smith, R. H. 1995. An in-vitro procedure to eradicate Potato viruses X, Y and S from Russet Norkotah and two of its strains. *In-vitro Cell Development Biology* 31: 153-159.