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DISTRIBUTION OF POTATO VIRUSES IN UGANDA

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ABSTRACT

Viral diseases are among the major factors affecting potato (*Solanum tuberosum L.*) production in Uganda. Knowledge of the types of viruses and their distribution levels in the country is vital for targeting sound management strategies. The objective of this study was to assess the prevalence and distribution of potato viruses in different potato growing areas in Uganda. Virus diagnostic surveys were conducted across the main potato producing regions and virus detection was done using DAS-ELISA and RT-PCR techniques. The study showed that the most widely distributed and economically important potato viruses were: PVS (31-90.9%), PLRV (2.6-81.3%), PVY (7.1-54.5%) and PVX (8.3 - 53.3%); while the least detected viruses were PVM (18.2%) and PVA (9%). The viruses were prevalent either singly or in mixtures. Up to 42.8% of the samples were infected with one virus, 20.9% with two viruses and 4.2% with three viruses; while no virus was detected in 31.3% of the samples. Of the double infections, the PVY+PVS combination was the most common and widely distributed (2.1-18.2%) in 12 districts; followed by PVY+PLRV (1.8-21.3%) occurring in six districts, PVM+PVS (7.9-16.7%), PLRV+PVX (2.4-14.3%) in 5 districts and PVY+PVX (2.4-4.4%) in 3 districts. Triple infections involving PVY+PLRV+PVS were recorded at prevalence levels of 2.2-18.6% in six district. Altitude, temperature, varieties and seed sources showed relationships with the variation in the prevalence of the viruses. There were high virus prevalence and disease severity levels in low altitude areas (1088-1334 m.a.s.l) of mid-western sub-region (Mbarara and Lwengo), Central (Kibaale, Mubende) and Mid Northern (Pader) sub region compared to high altitude areas (>1600 m.a.s.l). The Virus risk Area Modeling results showed the largest area (48.6%; 1,308,160 ha) at moderate risk of virus infection; while 27.3% (732,305 ha) was at high risk of virus infection. Based on the distribution level of the viruses, potato production could be intensified in areas with less virus pressure mainly, in parts of West Nile and Rwenzori regions.

Key Words: DAS-ELISA, *Solanum tuberosum*

RÉSUMÉ

Les maladies virales sont l'un des principaux facteurs affectant la production de pommes de terre (*Solanum tuberosum* L.) en Ouganda. La connaissance des types de virus et de leurs niveaux de distribution en Ouganda est essentielle pour cibler des stratégies de gestion rationnelles. L'objectif de cette étude était d'évaluer la prévalence et la distribution des virus de la pomme de terre dans différentes zones de culture de la pomme de terre en Ouganda. Des enquêtes de diagnostic viral ont été menées dans les principales régions productrices de pommes de terre et la détection des virus a été effectuée à l'aide des techniques DAS-ELISA et RT-PCR. L'étude a montré que les virus de la pomme de terre les plus largement répandus et les plus importants sur le plan économique étaient: PVS (31-90,9%), PLRV (2,6-81,3%), PVY (7,1-54,5%) et PVX (8,3% -53,3%); tandis que les virus les moins détectés étaient le PVM (18,2%) et le PVA (9%). Ces virus étaient répandus seuls ou en mélanges. Jusqu'à 42,8% des échantillons étaient infectés par un virus, 20,9% par deux virus et 4,2% par trois virus; alors qu'aucun virus n'a été détecté dans 31,3% des échantillons. Parmi les doubles infections, la combinaison PVY + PVS était la plus courante et la plus répandue (2,1 à 18,2%) dans 12 districts; suivi de PVY + PLRV (1,8-21,3%) survenant dans six districts, PVM + PVS (7,9-16,7%), PLRV + PVX (2,4-14,3%) dans 5 districts et PVY + PVX (2,4-4,4%) dans 3 les quartiers. Des infections triples impliquant PVY + PLRV + PVS ont été enregistrées à un niveau de prévalence de 2,2 à 18,6% dans six districts. L'altitude, la température, les variétés et les sources de semences ont montré une relation avec la variation de la prévalence des virus. La prévalence du virus et la gravité de la maladie virale étaient élevées dans les zones de basse altitude (1088-1334 m d'altitude) de la sous-région du centre-ouest (Mbarara et Lwengo), du centre (Kibaale, Mubende) et du centre-nord (Pader) par rapport aux zones de haute altitude (> 1600 m d'altitude). Les résultats de la modélisation des zones à risque viral ont montré que la plus grande zone (48,6%; 1 308 160 ha) présente un risque modéré d'infection virale; tandis que 27,3% (732 305 ha) étaient à haut risque d'infection virale. Sur la base du niveau de distribution des virus, la production de pommes de terre pourrait être intensifiée dans les zones où la pression virale est moindre, principalement dans certaines parties des régions du Nil occidental et de Rwenzori.

Mots Clés: DAS-ELISA, *Solanum tuberosum*

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the fourth most important food crop world wide, after rice wheat and maize in terms of human consumption (Zaheer and Akhtar, 2016). In Uganda, potato occupies the 8th position as a food security and a cash crop (Mbowa and Mwesigye, 2015). The productivity of potatoes at farm level in Uganda is estimated at 7.1 t ha^{-1} (FAOSTAT, 2016), which is rated very low compared to 25 t ha^{-1} achievable on research station under good management (Harahagazwe *et al*., 2018). Viral diseases contribute 10-100% of the yield losses in potatoes and also play a big role in seed degeneration by reducing the seed tuber quality (KAZARDI, 2016). The majority of smallholder potato farmers in Uganda use seed from the informal sector, in which seed-borne viruses accumulate over time (Priegnitz *et al*., 2019).

About 40 virus species have been reported to infect potato worldwide (Valkonen, 2007), but some of the viruses are restricted to certain geographical regions, while others occur worldwide (Kreuze *et al*., 2020).

Valkonen *et al.* (2015) indicated existence of six potato viruses with high incidences in Tanzania; namely, *Potato leafroll virus* (PLRV), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus Y* (PVY) and *Potato virus X* (PVX). Similar viruses were reported in Kenya by Okeyo (2017). The prevalence levels of these viruses varies depending on the mode of transmission, management practices,

temperature and presence of vectors (Jeger *et al*., 2004) as most of the viruses are transmitted by aphids and infected seed tubers (Brunt, 2001).

The severity of viral diseases is reported to be extremely variable in most potato fields, depending on weather conditions during the growing season (Madden and Nutter1995). The occurrence of six viruses in neighbouring countries, together with poor quarantine measures, poor vector control strategies which allow the vectors to move across borders, uncontrolled seed sales and movements with limited supply of certified seed in the country indicated a high possibility of having all these viruses widely distributed in Uganda. The objective of this study was to establish the prevalence and distribution of different potato viruses occurring in Uganda with a view to providing a basis for targeting management strategies.

MATERIALS AND METHODS

Sample collection. A diagnostic disease survey was conducted in the main potato production districts (Kabale, Rukiga, Rubanda, Kisoro, Rukungiri, Kapchorwa, Bulambuli, Kabarole, Kyenjojo, Zombo, Mubende, Kibaale, Pader, Mbarara and Lwengo) of Uganda during January - June 2018.

The districts surveyed were selected based on history of potato production in Uganda (Ferris, 2003) and UBOS (2010) potato production report. A total of 454 potato leaf samples were collected from 198 potato fields in districts across different agro-ecological zones. A multi-stage random sampling technique was used in the selected districts, targeting high, medium and low altitude areas according to the procedure of Levy and Lemeshow (2013). From each district, the main potato growing sub-counties were identified with help of the district production staff. Potato fields were selected based on distance and varied from 2-15 Km from each other, based on the assumption that fields near each other had similar virus infections as a result of seed saving and the intensity of potato production in the area.

From each selected field, the sampling process targeted potato plants showing various types of virus-related symptoms, such as chlorosis, yellowing, mosaic, necrosis, leaf rolling, stunted growth, systemic vein clearing, mottling, curling, shortening of leaves, dark green and vein-banding in the field and non-symptomatic samples. During surveys, virus suspected plants were photographed and symptoms of plants recorded. Leaf samples from the top, middle and lower parts of the plant were collected, labelled and placed in plastic bags, which were kept in a cooler box at 4 ^{o}C and delivered to the laboratory, where they were kept at -80 ^{o}C to prevent tissue degradation until the analysis was done. Other information that was gathered during the survey included the name of the variety, stage of growth at the time of sampling, and GPS location for every field. Potato variety names and seed sources were provided by the field owners, and verified for correctness with variety descriptors provided by the Uganda National Potato Breeding Programme.

Disease incidence and severity. To estimate the virus disease incidence in the field, a systematic X sampling pattern (Cooke, 2006) was followed and the disease status recorded for 100 plants along each of the four arms occupying quadrats of 10 m by 10 m area of the field. Field virus disease incidence was determined as a percentage of visually apparent symptomatic virus diseased plant over the total number of plants assessed (Madden *et al.,* 1999) and expressed as percentage per field; where by 1% unit represented one diseased plant out of 100 plants recorded in the field. Virus disease severity, on the other hand, was scored based on visual estimate of the disease symptoms of the foliage in the field for the 100 plants assessed using a scale of 1-5 according to Islam *et al.* (2015) where; 1 = no symptoms and 5 = very severe symptoms.

From each of the plants selected, two leaves from lower, middle and upper parts from

diseased potato plants and 1 symptomless plant were collected as samples and taken to the laboratory for identification of the viruses.

DAS-ELISA detection for the potato viruses. Viruses were detected using Double-Antibody Sandwich Enzyme linked Imunosorbet Assay (DAS-ELISA) kit acquired from International Potato Centre (CIP), Lima Peru for six major potato viruses (PVY, PLRV, PVX, PVS, PVA and PVM). Six leaf discs from leaf samples were grounded in 4 ml of PBST containing 2% (wt/vol) Polyvinyl pyrrolidone and the viruses were detected following the procedure described by Clark and Adams (1977). The optical density (OD_{405} nm) value was measured to determine the concentration/ titre of virus in infected potato samples using a microplate reader (Labdetect 96) that used capture 96 software (Kohl and Ascoli, 2017) and the samples were considered positive for the virus if their mean absorbance reading was twice that of the negative control.

RT-PCR detection for the potato viruses

RNA extraction. Total RNA was extracted from both virus infected positive and negative samples identified by DAS-ELISA using *AccuZol*™ reagent sourced from Bioneer Inc, according to manufacturer's instruction. About 0.5 g of the leaf tissues was ground in 1.5 ml eppendorf tubes using micro-tube sample pestles and liquid nitrogen (Vincelli and Amsden, 2013). Then, 1000 µl of *AccuZol*™ was added to the ground tissues in the tubes. The mixture was vortexed and left to stand at room temperature for five minutes. The mixture was then spinned at 12,000 rpm for 10 minutes at 4 °C. The supernatant was removed and put in to a new tube containing 600 µl of chloroform. The mixture was shaken by hand for 15 seconds and then left to stand for 15 minutes.

The mixture was again spinned at 12,000 rpm for 15 minutes at 4 °C after which the aqueous layer was transferred to a new tube. Then 600 µl of absolute Iso-propanol was added and allowed to stand for 10 minutes. The mixture was spinned at 12,000 rpm for 10 minutes at 4 °C. The supernatant was then aspirated without disturbing the pellet. The pellet was washed with 1000 µl of 70% ethanol and the supernatant was again aspirated without disturbing the pellet. The pellet was then dried for 10 minutes at room temperature and then dissolved in 50 µl of RNase free water.

cDNA synthesis and virus detection. Reverse transcription polymerase chain reaction (RT-PCR) was done according to Crosslin and Hamlin (2011). The resulting cDNA from RT was used as a template for PCR. PCR reaction mixture contained 3 µl of the cDNA template, 0.2 mMdNTPs, 2.5 µl of 10X Polymerase buffer, 2.5 µl of 25 mM Mgcl2, 1 µl of 10 µm of virus forward and reverse primer (Table 1), 0.5 µl of 10 mMdNTP mix and 1 unit of the Taq DNA polymerase.

The reaction mixture was amplified by 35-40 cycles in a thermocycler, following standard procedure with annealing temperature determined prior for each virus using a gradient PCR. Each cycle consisted of initial denaturation at 95 °C for 2 minutes followed by annealing step at 56.9 °C (PVY), 55 °C (PLRV), 55 °C for PVS, 57 °C for PVX for 30 seconds and extension at 72 °C for 1 minute and 30 seconds. The PCR products were separated by electrophoresis on 1.5% agarose gel that was stained with ethidium bromide and the product of electrophoresis was visualised and photographed under UV light (Westermeier, 2016).

Meteological data for the areas sampled. Weather data for the period of January - June 2018 during the study were obtained from the Uganda National Meteological Authority (UNMA) for districts where samples were collected. The information obtained included daily rainfall, temperature and humidity (Fig. 1).

TABLE 1. Primers for RT-PCR detection of potato viruses

Virus	Primer sequences		Product size (base pairs)	Reference
	Forward primer 5' -3'	Reverse primer 5' -3'		
PVA	GTACTGAACTGGAAAAGTACT	CCCTGACAGTTGAAACATAA	~1,100	Rajamäki *et al.*, 1998
PVM	GCCACATCYGAGGACATGAT	GTGAGCTCSGGACCATTCAT	524	Crosslin *et al.*, 2011
PVS	GAGGCTATGCTGGAGCAGAG	AATCTCAGCGCCAAGCATCC	738	Crosslin *et al.*, 2011
PVX	TAGCACAACACAGACCACAG	GGCAGCATTCATTTCAGCTTC	562	Nie and Singh, 2001; Bostan and Peker, 2009
PVY (all strains)	CCTCCTTCTCTGAAAGGTGAT	TGCCAAAGCTTGGAACCTGG	450	Schubert *et al.*, 2007
PLRV-1	CGCGCTAACAGAGTTCAGCC	CCAAATACTACTTTAACCGCA	466	Singh, 1999
PLRV-2	TAGCATGCCAGTGGTTATGGTC	GCCTCGAGTCTACCTATTTGG	534	Querci *et al.*, 1997

Data analysis

Prevalence of potato viruses. Incidences of individual potato viruses were calculated on district and regional basis using the number of samples that tested positive for the presence of the viruses. The Generalised Linear Module (GLM) of the GenStat was used to analyse and interpret the data, using statistical package GenStat (VSN International Ltd. UK) (Payne, 2009). Analysis of variance (ANOVA) results were used to calculate least significant differences (LSD) between mean of prevalence values. For comparing separated means, LSD and pairwise comparisons test were carried out at a probability level of $P<0.05$ (Williams and Abdi.2010).

Virus risk area analysis. Interpolation of factors that influence risk of infection which included incidence, severity, altitude, average temperature and rainfall, was done using geospatial analysis package of Arch Map using IDW (Inverse distance weight) to map out virus risk areas (Belief, 2018). Inverse distance weighted (IDW) was used because of its potential to predict the values for any unmeasured location, by measuring the surrounding values from predicted location (Childs, 2004) based on two assumptions: first, the influence of un- known value of a point is increased directly to the close control point than far points. Secondly, the influence degree point is directly proportional to the inverse of the distance between points. It was based on the following equation (Bartier and Keller, 1996).

$$Z = \frac{\sum_{i=1}^{n} w_i Z_i}{w_i} = \frac{\sum_{i=1}^{n} Z_i / D_i^p}{1/D_i^p}$$

Where:

Z refers to the interpolated value of unknown point, is the weighting function that control the significance of control point and is the observed value at the control point i which

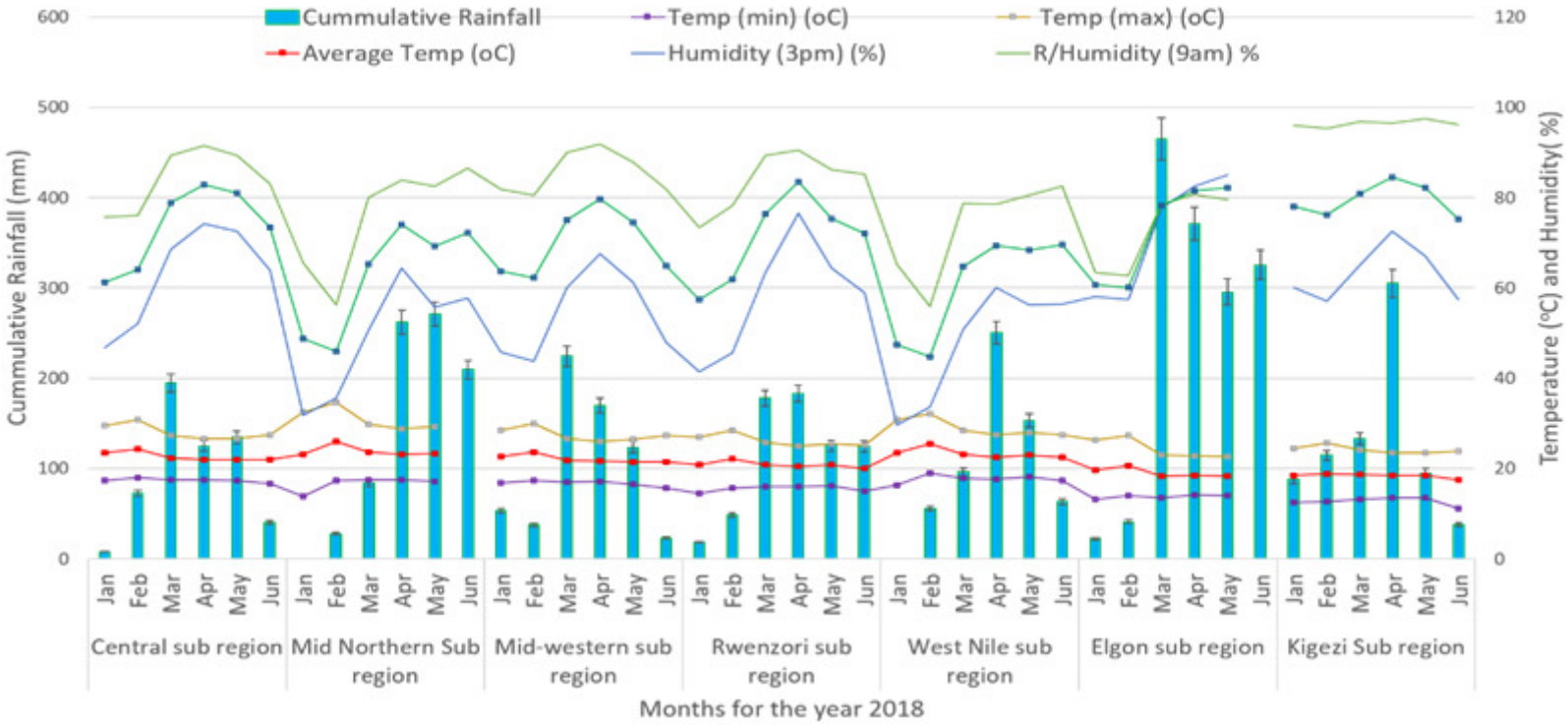

Figure 1. Humidity temperature and Rainfall distribution pattern for the sampled study areas during 2018. Source: Uganda National Meteorological Authority.

represent the nearest neighborhood of produced interpolated point, n is the nearest neighborhood of control points that is usually required time consummation. D^p_i refer to the distance between I and interpolated point, p is a weighting exponent which is an arbitrary positive real number, p is equal to 1 in inverse distance weighting (Guan and Wu, 2008).

Interpolation used the measured values recorded in the field surrounding the point location to predict the values for any sample based on assumption that samples that were close to one another were more alike than those far apart (Hartkamp, 1999). Raster layers for the virus severity, incidence, altitude, temperature and rainfall data were created in a raster and converted to raster's images (30 m resolution) and classified to represent the distribution of the of the virus infection in the potato growing areas and given a scale of 1-3 where 1 = low risk, 2 = moderate risk and 3 high risk. The maps representing the effective weights of main factors which are the disease incidence, disease severity altitude were combined to give effective one disease risk potential prediction map under each risk level (1-3) based on Nelson *et al*. (1999) and area (ha) of each category was computed from the GIS.

RESULTS

Viruses infecting potatoes in Uganda. Laboratory analysis of the symptomatic and asymptomatic samples using DAS-ELISA and RT-PCR techniques showed that six viruses (PVS, PLRV, PVY, PVX, PVM and PVA) were the main viruses infecting potatoes in Uganda (Table 2). PVS was the most prevalent virus among those tested and was detected in 43.8% out of 454 samples; while PLRV was detected in 26.4% of the samples, PVY (17.8%), PVX (15.4%), PVM (3.5%) and PVA (0.9%).

Viruses occurred as single infection and in mixed infections. Single virus infection were observed in 42.8% of the samples, double infection was recorded in 20.9% of the samples; triple infection was noted in 4.2% of the samples while quadruple infection was noted in 0.9% of the samples. There was no

TABLE 2. Potato viruses detected in symptomatic and asymptomatic leaf samples collected from various parts of Uganda

Virus infection in symptomatic and asymptomatic samples	Number of positive samples	Percentage infection
PVS	199	43.8
PLRV	120	26.4
PVY	81	17.8
PVX	70	15.4
PVM	16	3.5
PVA	4	0.9
Single virus infections	194	42.8
Double virus infection	95	20.9
Triple virus infection	19	4.2
Quadruple infections	4	0.9
Positive samples (Symptomatic)	276	60.8
Positive samples (Asymptomatic)	36	7.9
Negative samples (symptomatic)	58	12.8
Negative samples (Asymptomatic)	84	18.5
Total number of plant samples tested	454	

virus detection in 12.8 and 18.5% of the symptomatic and asymptomatic samples, respectively; and yet some of the symptomatic samples had expressed clearly virus-like symptoms and were suspected to have been carrying some viruses. Molecular detection using RT-PCR technique revealed more samples that had previously tested negative with DAS-ELISA especially for PVX (Fig. 2).

RT-PCR detection of different viruses using specific primers for the virus coat protein produced the expected amplicon of 450bp for PVY, PLRV (466bp), PVX (562bp) and PVS (738bp). The primers for PVM and PVA did not amplify the template cDNA for PVM and PVA although DAS-ELISA tests indicated their presence, indicating a need to use more virus specific primers for these viruses.

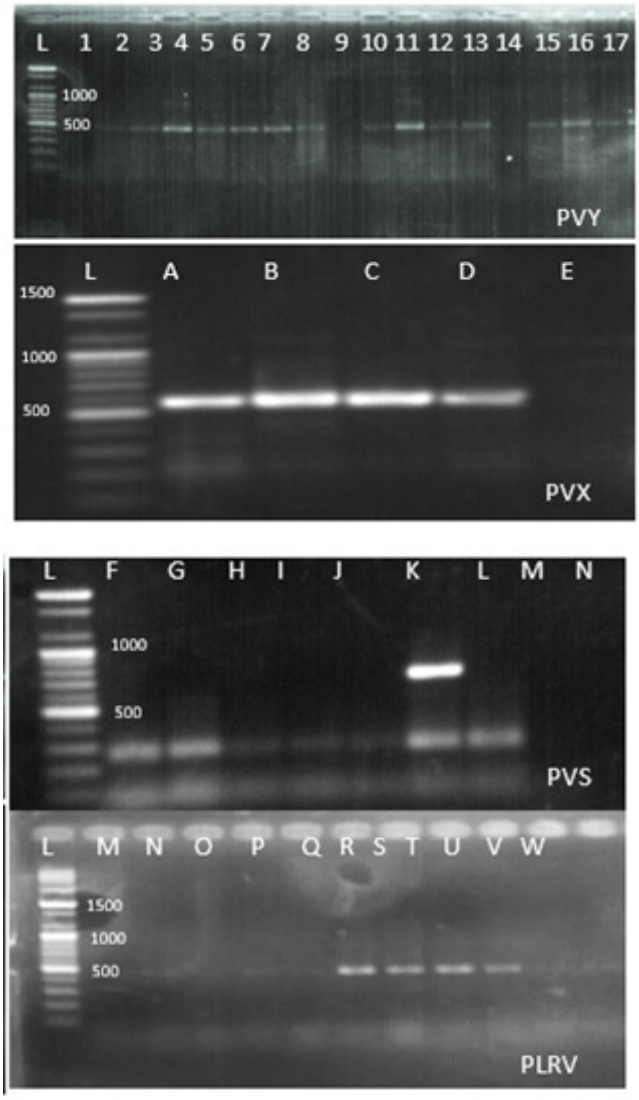

Figure 2. RT-PCR detection of PVY, PVX, PVS and PLRV viruses in infected potato leaf samples. L=100 bp ladder, samples 1-17, positive samples for PVY and 9 is negative for PVY, A-D, are positive for PVX virus, E is negative control for PVX, K is positive for PVS strain O, F and G are positive for PVS strain A. Sample R S, T and U are positive for PLRV, O was negative control of PLRV.

Prevalence and distribution of potato viruses. The prevalence levels (%) and distribution of each virus in the areas surveyed was found to be variable (Fig. 3).

PVS was the most common with prevalence range of 31-90.9%. The highest prevalence was recorded in Lwengo (90.9%); followed by Rukinga (83.3%) and was lower in Kisoro (16.7%), but was not observed in Kyenjojo district.

PLRV was the second predominant virus, with higher prevalence rates in Kisoro (81.3%) and Kapchorwa (55%) and lower prevalence in Mbarara (15.6%), Mubende (9.1%), Zombo (7.3%) and Bulambuli (2.6%). However PLRV was not detected in Rukungiri.

PVY was the third most common virus. The prevalence of PVY differed significantly among the districts. Greater prevalence levels of PVY were recorded in the districts of Lwengo (54.5%), Kisoro (35.4%), Mbarara (33.3%), Rubanda (29.7%), whilst there was significantly lower prevalence in Bulambuli (18.4%), Zombo (12.7%), Kabale (10.2%), Rukungiri (8.3%) and Rukiga (7.1%).

PVX was the fourth most prevalent viruses. The prevalence of PVX varied significantly across the districts and within the sub regions and the highest prevalence was recorded in Kisoro (54.2%) and Mbarara (53.3) and least in Kabarole (8.3%), Kibaale (6.3%) and Kabale (5.1%).

The prevalence of PVM and PVA was lower than that of PVS, PLRV, PVY and PVX. The PVM was found more in the districts of Mubende (18.2%), Rukungiri (16.7%), Kabarole (16.7%), but less in Zombo, (5.5%) and Mbarara (2.2%). However, PVM was not detected in Kabale Kisoro, Rubanda, Rukiga and Kyenjojo districts. PVA was the least detected among the viruses in all the areas, and was found only in Lwengo at prevalence rate of (9.1%), Rukungiri (8.3%) and Rubanda (5.4%).

Correlation analysis of the prevalence levels of the viruses with the environmental factors showed that altitude, temperature (Maximum) and accumulated rainfall were the most important factors that significantly ($P<0.05$) influenced the variability in the prevalence and distribution of the viruses(Table 3).

Altitude contributed positively to the variations in the prevalence of PLRV($r= 0.545$) compared to other viruses PVY, PVS, PVX and PVA. Accumulated rainfall explained significantly the variation in prevalence of PVY ($r=0.536$) and PLRV ($=0.846$). Humidity when correlated with the prevalence of individual viruses, incidence and disease severity explained less of the variation.

Regression analysis of environmental factors that correlated with the prevalence of viruses revealed that maximum temperature, altitude, cumulative rainfall and seed sources

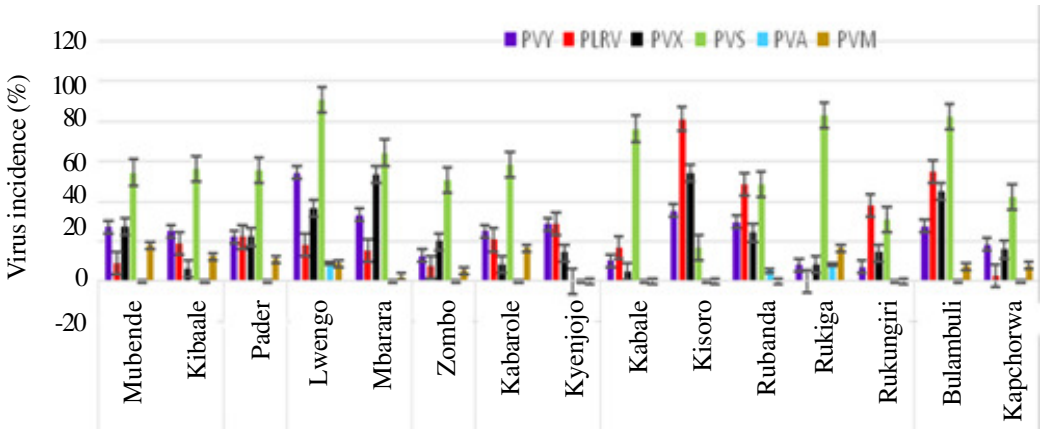

Figure 3. Prevalence (%) of virus across the major potato growing districts of Uganda.

TABLE 3. Correlation matrix between the environmental conditions and prevalence of viruses in major potato growing areas in Uganda

	Humidity	Cum Rainfall	Temp min	Temp max	Min-Altitude	Max-Altitude	Prevalence of viruses					
							PVY	PLRV	PVX	PVS	PVA	PVM
Humidity (%)	1											
Cumulative rainfall (mm)	0.059	1.000										
Temp min (°C)	-0.676	-0.358	1.000									
Temp max (°C)	-0.588	-0.345	0.869	1.000								
Min-Altitude (M.asl)	0.528	0.462	-0.716	-0.898	1.000							
Max –Altitude (M.asl)	0.594	0.438	-0.644	-0.802	0.885	1.000						
PVY	0.149	0.536	-0.101	-0.188	0.200	0.361	1.000					
PLRV	0.295	0.846	-0.555	-0.632	0.642	0.546	0.624	1.000				
PVX	-0.079	0.138	0.318	0.127	0.121	0.252	0.074	0.038	1.000			
PVS	-0.057	0.100	0.254	0.150	-0.007	0.037	-0.142	-0.056	0.017	1.000		
PVA	0.228	-0.156	0.110	-0.072	0.144	0.033	-0.168	-0.029	-0.011	0.486	1.000	
PVM	-0.233	-0.059	0.190	0.434	-0.492	-0.485	-0.318	-0.205	-0.271	0.455	0.191	1.000

were significantly (P<0.05) associated with the prevalence and distribution of the viruses in the areas where the potatoes were growing and results are presented in Table 4.

Potato fields that had used poor quality seed (home saved) were associated with high prevalence of virus infections, as opposed to those fields that had used quality seed as indicated by the negative regression coefficient (-4.14417). Areas within the high altitudes areas and those that received high amounts of rainfall (mm) were associated with low prevalence of viruses, compared to those located in the low altitudes and with relatively low amount of rainfall as indicated by the negative regression coefficient values.

Despite the wide scale distribution of the PVS, PLRV, PVY, PVX and PVM observed in this study, the laboratory results showed occurrence of mixed virus infections across the 15 district surveyed, with non-uniform distribution, and the results of the prevalence rates are presented in Figure 4.

The mixed viral infection involving PVY+PVS was the most common and widely distributed in 12 out of 15 districts. The prevalence of PVY+PVS ranged from 2.1 - 18.2%, with Mubende district registering the highest level of 18.2%, followed by Kibaale (12.5%), Kapchorwa (12.5%), Pader (11.1), Lwengo (9.1%), Zombo (5.5%), Rubanda (5.4%), Bulambuli (5.3%), Kabarole (4.2%), Kabale (3.4%), Mbarara (2.2%), Kisoro (2.1%). However it was not found in Kyenjojo and Rukiga districts.

The second most common mixed virus infection was the one involving PVY+PLRV with prevalence rate of 1.8-21.3%. It was recorded in 3 sub regions (Kigezi, Elgon and west Nile) in six districts, but mostly in Kigezi sub region in the districts of Kisoro (21.3%), Rubanda (10.8%), Rukiga (7.1%). However, it was not found in Central and Rwenzori sub region.

PLRV+ PVS was third most prevalent mixed infection observed in seven district mainly; Kapchorwa at prevalence level of 12.5%, Pader (11.1%), Rubanda (8.1%), Rukiga (7.1%), Kabarole (4.2%), Kisoro (4.2%) and Kabale (3.4%). Despite of its wide distribution covering most of key main potato growing districts, it was not found in the Central sub region and Mid-western sub region.

The prevalence of mixed infection involving PLRV+PVX ranged from 2.4-14.3% and were observed in 5 districts of Kyenjojo (14.3%), Kisoro (10.4%), Kibaale (6.3%), Kabarole (4.2) and Rukiga (2.4%). PVM+PVS had prevalence level in Kabarole of 16.7%, Rukungiri (16.7%), Kibaale (12.5%), Mubende (9.1%), Bulambuli (7.9%) Kapchorwa (2.5%), Zombo (1.8%). Virus infection involving PVY+PVX was observed in Mbarara (4.4%), Kabale (3.4%) and Rukiga (2.4%).

Triple virus infection involving PVY+PLRV+PVS was noted in six district of Lwengo with a prevalence of 18.2%, Rubanda (10.8%), Kapchorwa (5%), Kabarole (4.2%), Kisoro (4.2%) and Mbarara (2.2%) while that

TABLE 4. Regression results for the factors influencing distribution of viruses

	Coef.	Std. Err	T	P>|t|	Beta
Altitude (m.asl)	-0.01462	0.00432	-3.38	0.001	-0.32634
Temp max (°C)	-3.40303	1.529534	-2.22	0.027	-0.40805
Temp min (°C)	1.534091	1.623759	0.94	0.346	0.179674
Cumulative rain fall (mm)	-0.01417	0.00356	-3.98	<0.001	-0.27897
Seed quality	-4.14417	1.499453	-2.76	0.006	-0.11792
Constant	142.383	27.93689	5.1		

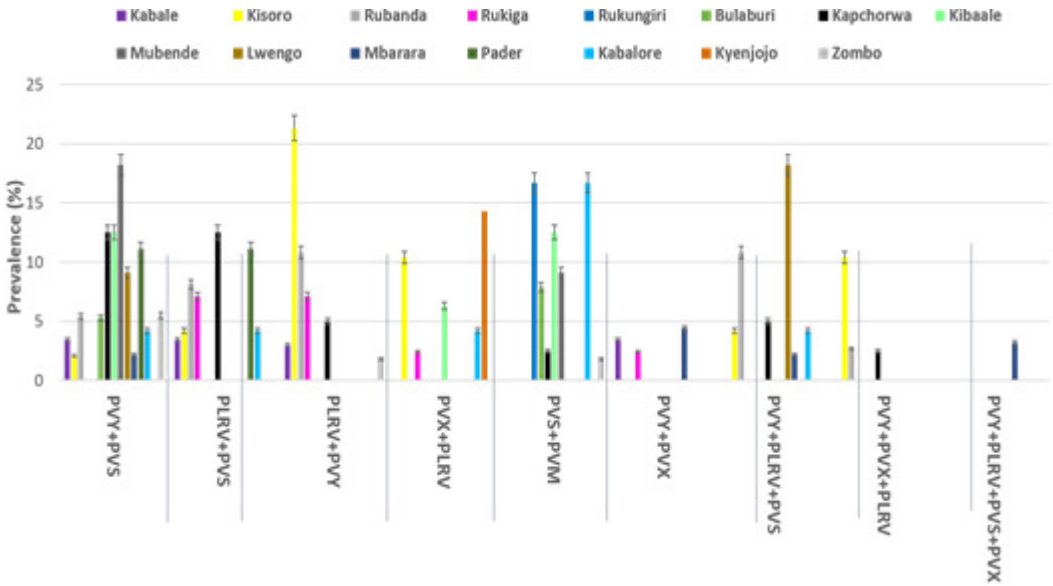

Figure 4. Distribution of mixed virus infections in the potato growing districts in Uganda.

of PVY+PVX+PLRV was observed in Kisoro at a prevalence of (10.4%), Rubanda (2.7%) and Kapchorwa (2.5%). Quadruple infection involving PVY+PLRV+PVS+PVX was only observed in Mbarara with a prevalence of 3.2%.

Incidence and severity of the virus diseases. The results showed significant (P<0.05) variation in the incidence and severity of virus diseases across the altitude range of 1085-2427 m.asl. The highest disease incidences were recorded in districts of Lwengo (65.18%); followed by Kyenjojo (60.54%), Mbarara (58.30%) and lowest in Kabarole (3.32) and Rubanda (3.11) (Table 5). These viruses had severity that ranged from 2.7 - 4.2.

Regression analysis of the factors that was associated with severity of viral disease showed that virus disease severity was significantly (P<0.05) influenced by the type of the virus, number of viruses infecting the plants and the stage of growth at the time of sampling (Table 6).

The results showed that some viruses such as PVY, PLRV and PLRV+PVY contributed significantly (P<0.05) to the disease severity in the field. The overall F statistics and the t test of estimated parameters were significant at $P < 0.05$ as indicated by the regression analysis. A significant interaction (P<0.05) was also observed between the disease severity, type of virus and the growth stage of the plant. Higher disease severity was recorded for plants between flowering stage to full canopy size compared to plants which were had matured.

Potato varieties, seed sources and virus distribution. The distribution of the varieties which were identified to have been infected with the viruses, was not uniform across districts with some limited to specific regions while other were widely distributed across all the sub regions studies (Table 7)

The six varieties were observed to be widely distributed across the growing regions. Rwashaki variety was found only in Kigezi sub region with distribution level in the field at 10.1-32%. Variety Kinigi was found only in Kigezi and Elgon sub region and was the dominant variety in Kisoro at 66.7%; while Victoria was found in six sub regions, except the mid North (Pader). Variety Rwagume was the most commonly and widely distributed across all

TABLE 5. Viral disease incidence and severity across altitudes in the major potato growing areas in Uganda

Sub region	District	Min-Altitude (m.asl)	Max-Altitude (m.asl)	Temp min °C)	Temp max (°C)	Temp (mean) (°C)	Cumulative rainfall (mm)	Humidity (%)	Fields	Viral disease incidence	Disease severity
Central	Mubende	1,274	1,298	17.37	26.2	23.1	575.2	73.6	15	56.93 ± 10.73	4.21 ± 0.44
	Kibaale	1,088	1,327	17.37	26.2	23.1	575.2	73.6	12	45.48 ± 4.56	4.16 ± 0.34
Mid Northern	Pader	1,085	1,116	16.68	27.95	24.1	854.8	62.6	9	48.29 ± 6.58	4.18 ± 0.21
Mid-western	Mbarara	1,344	1,876	16.78	26.25	23.3	632.3	70	33	58.32 ± 14.07	3.78 ± 0.75
	Lwengo	1,266	1,323	16.78	26.25	23.3	632.3	70	15	65.21 ± 5.06	3.69 ± 0.9
West Nile	Zombo	1,515	1,659	17.68	22.3	20	619	60.7	25	16.75 ± 3.74	3.49 ± 0.4
Rwenzori	Kabarole	1,135	1,654	15.56	24.2	19.9	677.8	71.1	15	14.64 ± 5.68	3.59 ± 0.45
	Kyenjojo	1,262	1,338	15.56	24.2	19.9	677.8	71.1	13	60.5 ± 10.36	3.95 ± 0.44
Kigezi	Kabale	1,820	2,284	12.76	21.4	17.1	773.7	79.5	26	40.53 ± 6.12	2.76 ± 0.7
	Rukungiri	1,640	1,709	17.8	25.4	21.6	773.7	79.5	10	38.54 ± 4.43	3.17 ± 0.55
	Rukiga	1,730	1,918	12.76	21.4	17.1	773.7	79.5	15	30.21 ± 3.76	3.59 ± 0.5
	Rubanda	1,939	2,427	12.76	21.4	17.1	773.7	79.5	15	33.07 ± 3.22	2.97 ± 0.6
	Kisoro	1,808	2,374	12.76	21.4	17.1	773.7	79.5	26	45.4 ± 6.64	2.97 ± 0.86
Elgon	Kapchorwa	1,755	2,210	13.8	22.25	18	1520.8	72.5	25	32.08 ± 6.33	3.06 ± 0.82
	Bulambuli	1,802	2,072	13.8	22.25	18	1520.8	72.5	20	28.42 ± 6.96	3.51 ± 0.52
F.pr										<.001	<.001
L.s.d (0.05)										5.847	0.329

Meteorological data (Temperature, Rainfall, Humidity) was for January 2018-June 2018 and was sourced from Uganda National Meteorological Authority

TABLE 6. Regression results of factors influencing virus disease severity in major potato frowing areas in Uganda

Factors for disease severity	Mean	Coef.	Std. Err	T	P>|t|	Beta	95% CI
Type of virus							
PVY	3.84	0.486	0.137	3.55	<0.001	0.293	0.22, 0.76
PVX	3.55	0.341	0.203	1.68	0.094	0.196	-0.06, 0.74
PVS	3.18	-0.103	0.162	-0.63	0.527	-0.067	-0.42, 0.22
PVA	2.37	0.499	0.398	1.25	0.211	0.078	-0.29, 1.28
PVM	3.57	0.769	0.319	2.41	0.056	0.236	0.14, 1.40
PLRV	3.81	0.307	0.152	2.02	0.045	0.187	0.01, 0.61
PVY+PVS	3.75	0.238	0.274	0.87	0.387	0.064	-0.30, 0.78
PVY+PLRV	4.08	0.328	0.154	2.14	0.034	0.138	0.03, 0.63
PVM+PVS	3.66	-0.1	0.398	-0.25	0.801	-0.025	-0.88, 0.68
PLRV+PVX	3.19	-0.286	0.254	-1.13	0.261	-0.08	-0.79, 0.21
PVY+PVX	3.86	-0.064	0.422	-0.15	0.879	-0.009	-0.90, 0.77
PVY+PVX+PLRV	4.01	-0.587	0.385	-1.52	0.129	-0.092	-1.35, 0.17
PVY+PLRV+PVS	4.2	0.075	0.381	0.2	0.844	0.012	-0.67, 0.83
Number of viruses							
Single	3.38	-0.359	0.125	-2.87	0.004	-0.229	-0.61, -0.11
z							
Double	3.71	-0.511	0.185	-2.76	0.006	-0.292	-0.88, -0.15
Triple	4.11	-0.77	0.242	-3.18	0.002	-0.31	-1.25, -0.29
Plant growth stage							
Flowering to full canopy cover	3.81	0.557	0.163	3.43	0.001	0.273	0.24, 0.88
Maturity	3.41	0.212	0.128	1.66	0.098	0.128	-0.04, 0.46
Constant		3.073	0.177	17.35	0	.	2.72, 3.42

TABLE 7. Distribution of varieties in the main potato growing regions in Uganda and their seed sources

Sub region	District	Potato fields sampled	Variety distribution (%) in the districts					% of the of potato fieldsutilising the seed sources			
			Kinigi	Rwagume	Rwashaki	Victoria	Kimuri	New Dutch	Home saved	Seed multiplier	NARO
Kigezi Sub region	Kabale	20	20.3	59.5	10.1	6.8	1.7	1.6	93.5	4.5	2
	Kisoro	20	66.7	16.7	16.6	0	0	0	95.6	2.4	2
	Rubanda	15	10.8	35.1	32.4	10.9	0	10.8	96	4	0
	Rukiga	16	9.5	61.9	0	19	4.8	4.8	96.2	3.8	0
	Rukungiri	10	0	91.7	0	0	8.3	0	100	0	0
Elgon sub region	Bulambuli	20	10.5	39.5	0	36.8	5.3	7.9	94.8	3.7	1.5
	Kapchorwa	25	0	90	0	10	0	0	97.8	2.2	0
Central region	Kakumiro	10	0	87.5	0	6.3	6.2	0	100	0	0
	Mubende	15	0	100	0	0	0	0	100	0	0
Mid-western sub region	Lwengo	15	0	100	0	0	0	0	100	0	0
	Mbarara	20	0	73.3	0	22.3	4.4	0	98.5	1.5	0
Mid-Northern sub region	Pader	8	0	100	0	0	0	0	100	0	0
Rwenzori Sub region	Kabarole	14	0	45.8	0	45.8	4.2	4.2	97.8	0	2.2
	Kyenjojo	13	0	71.4	0	28.6	0	0	100	0	0
West Nile sub region	Zombo	25	0	49.1	0	32.7	1.8	16.4	97.2	2	0.8

the districts, with distribution levels of 16.7%-100% across the districts. The new Dutch varieties were found in Kigezi, West Nile, Rwenzori and Elgon sub region, mainly around the research station, but with low distribution level (1.7 - 16.4%) with the highest (16.4%) observed in West Nile (Zombo). The results from the potato fields surveyed during the study showed that majority of fields (97.82%) had used home saved seed; while 1.6 and 0.5% used seed from seed multiplier and NARO research stations. There was significant variation ($P<0.05$) in the prevalence of each virus across the different varieties (Table 8).

The prevalence of viruses was highest in variety Rwagume for PVS (38.5%), followed by PVY (18.4%), PLRV (16.6%), PVX (15.8%), PVM (5.5), PVA (1.2%). Variety Rwashaki had virus prevalence level of (3.7-16.8%) with highest for PLRV (16.8%), PVS (13.6%), PVY (9.6), PVX (3.7%). Virus PVA and PVM were not detected on Rwashaki variety. The prevalence of viruses on variety Kinigi ranged from 6-13.2% with PLRV (13.2%), PVX (13.2%), PVS (6.4) and PVY (6%); while PVM and PVA were not detected in Kinigi variety. Victoria variety had all the six viruses infecting it with prevalence of 0.3-12.8%. The new Dutch varieties, which were found growing in fields around the research station, were observed to have low levels of virus prevalence with PVS at 3.5%, PLRV (1.6%), PVX (1.2%), PVY (0.5%) and PVA (0.5%); while PVM was not detected on the new Dutch. Among all varieties Rwagume and Rwashaki and were most affected by viruses followed by Kinigi and Victoria.

Mapping virus risk areas. The results for the modeling of areas at risk of virus infection are presented in Figure 5. The area with low risk of virus infection is indicated by green area, while those with moderate risk (yellow) and high risk (red). Risk mapping showed that significant difference in the percentage total area (ha) under risk of virus infection with 48.6% of the total area (ha) for all the districts growing potatoes at a moderate risk, 27.3% at a high risk, 24.1% at low risk (Table 9).

The largest % area (ha) with high risk of virus infection was observed in Mbarara (47 %, 86,762 ha), followed by Lwengo (45%; 41,161.5 ha), Kisoro (45%, 32,760.0 ha), Kyenjojo (40%, 94,000.0 ha), and least in, Kabarole (15%, 27,210.0 ha) and Zombo (1.165%, 1,041.7 ha).

The area (ha) with moderate risk of virus infection was observed highest in Pader (75%, 252,150.0 ha), followed by Lwengo (55% 50,308.5 ha), Rubanda (54%, 23,020.2 ha), and least in Zombo (27.73%, 24,886.77 ha). The area with low risk level were found highest in Zombo (71.11%, 63,831.5 ha), and Kabarole (45.0%, 81,630.0 ha).

TABLE 8. Prevalence of virus infection among the potato varieties in Uganda

Variety	Individual virus prevalence (%)					
	PLRV	PVX	PVY	PVS	PVA	PVM
Rwagume	16.6	15.8	18.4	38.5	1.2	5.5
Rwashaki	16.8	3.7	9.6	13.6	0.0	0.0
Kinigi	13.2	13.2	6.0	6.4	0.0	0.0
Victoria	1.8	3.5	3.1	12.8	0.3	1.7
Kimuri (Land rance)	0.8	0.3	0.8	3.2	0.0	1.0
New Dutch	1.6	1.2	0.5	3.5	0.5	0.0
F.pr	0.003	0.009	<.001	<.001	0.699	0.028
L.s.d	8.85	8.8	7.13	14.5	1.568	3.357

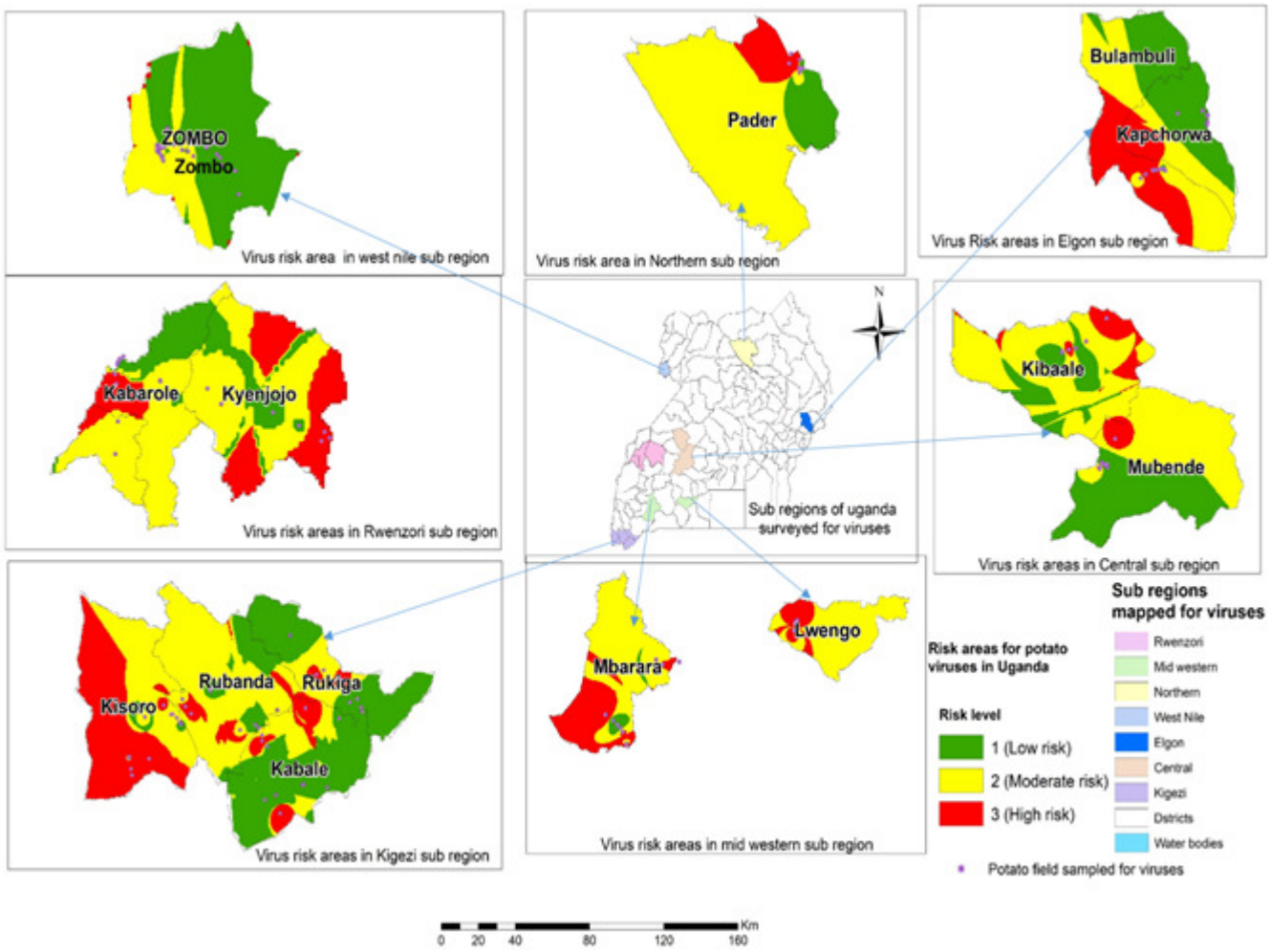

Figure 5. Modeled risk areas for potato virus diseases in Uganda.

TABLE 9. Modeled area (ha) for the districts growing potatoes and the associated risk of virus infections in Uganda

Sub region	District	Altitudes where potatoes were found		Temperature (°C)		Modeled % area (ha) at risk of virus infection			Computed total area (ha) at risk of virus infection		
		Mini-Altitude (Masl)	Max–Altitude (Masl)	Temp min (°C)	Temp max (°C)	Low risk (%)	Moderate risk (%)	High risk (%)	Low risk (ha)	Moderate risk (ha)	High risk (ha)
Central	Mubende	1,274	1,298	17.37	26.2	35	50	20	162,575	232,250	92,900
	Kibaale	1,088	1,327	17.37	26.2	15	50	35	76,515	255,050	178,535
Mid northern	Pader	1,085	1,116	16.68	27.95	10	75	15	33,620	252,150	50,430
Mid western	Mbarara	1,344	1,876	16.78	26.25	3	50	47	5,538	92,300	86,762
	Lwengo	1,266	1,323	16.78	26.25	-	55	45	0	50,309	41,162
West Nile	Zombo	1,659	1,659	17.68	22.3	71.11	27.73	1.16	63,832	24,887	1,042
Rwezori	Kabarole	1,135	1,654	15.56	24.2	45	40	15	81,630	72,560	27,210
	Kyenjojo	1,262	1,338	15.56	24.2	15	45	40	35,250	105,750	94,000
Kigezi	Kabale	1,820	2,284	12.76	21.4	40	30	30	67,160	50,370	50,370
	Rukungiri	1,640	1,709	17.8	25.4	35	45	20	50,572	65,021	28,898
	Rukiga	1,730	1,918	12.76	21.4	33	44	23	14,068	18,757	9,805
	Rubanda	1,939	2,427	12.76	21.4	26	54	20	11,084	23,020	8,526
	Kisoro	1,808	2,374	12.76	21.4	20	35	45	14,560	25,480	32,760
Elgon	Kapchorwa	1,755	2,210	13.8	22.25	40	40	20	14,184	14,184	7,092
	Bulambuli	1,802	2,072	13.8	22.25	25	40	35	16,295	26,072	22,813

DISCUSSION

Prevalence and distribution of potato viruses. The results showed that six potato viruses (PVS, PLRV, PVX, PVY PVM and PVA) were detected infecting potatoes (Table 2) and are widely distributed with varying prevalence levels across the major potato growing areas of Uganda (Fig. 3). The variation in prevalence was attributed to the influence of (i) altitude that could have impacted on the vector movement, (ii) temperature differences across the different sub region that could have influenced the susceptibility of the varieties, (iii) types of the variety grown by the farmers and (iv) seed quality that served as the source of virus inoculum. The least distributed viruses were PVA and PVM; although PVM and PVA were noted at low incidences, over time because of their nature of spread through seed and insect vectors, they may increase and contribute to greatlty to low potato production in Uganda.

The high number of viruses infecting potatoes with high prevalences in all the potato growing areas, suggest the role played by viral diseases in reducing potato productivity in the country. There may be more viruses infecting potatoes in Uganda, which have not been identified by the DAS-ELISA and RT-PCR approaches, which were available in this study as some of the symptomatic samples tested negative. This suggest a need to use other robust techniques such as deep sequencing platforms targeting small RNA profiles to unravel other viruses.

The viruses occurred as single and mixed infections with PVY+PVS, PVY+PLRV, PLRV+PVX PVY+PVX, and PVM+PVS constituting the majority (20.9%) of mixed infection; and those involving three or four viruses were detected in small proportions (5%) (Fig. 4). Similar results were reported by Valkonen *et al.* (2015) in Tanzania, where co-infections with PVS and PLRV were at 14% and mixed infection involving three or four viruses were at 5% of the tested plants. Yardýmcý *et al*. (2015) also reported mixed infection involving PVY+PVS (32.73%), PVX+PVS (5.39%), PVX+PVY (1.43%) and PVY+PLRV (1.05%) as most common in Turkey. The presence of mixed infections indicates a possibility of different potato viruses interacting in different manner (antagonism, synergism or neutral) to impact on the yield. The above results imply that the country stands a high risk of low potato production through virulent attack of mixed viruses which negatively affect production of the crop.

The virus prevalence levels of PVS, PLRV, PVX and PVY observed in this study compared well with studies in the rest of the world (Pourrahim *et al.*, 2007; Abbas *et al.*, 2012 and MacKenzie *et al.* 2018) where quality certified seed do not reach all farmers. In Kenya, Okeyo (2017) reported a higher level of prevalence of the viruses; PLRV, PVS, PVM and PVY with PVS being the most dominant virus (67%); followed by PVY (20%), PLRV (12%) and PVM (7%). In China, an average PVS infection level of 16.3%, reaching values of 22.6- 26.7% (Wang *et al*., 2011). In Costa Rica, Vásquez *et al*. (2006) reported incidence of PVS at 19%; while in in Iran PVS was reported at incidence of 18.2% (Salari *et al*., 2011).

The low prevalence levels for PVA observed in this study could be due to variety susceptibility differences to PVA infection or high infection levels of other viruses, such as PVS and PVY that could have interfered with establishment and replication of PVA as virus interference and cross protection to infection by PVA due to PVS and PVY and PVX have been demonstrated before (Lico *et al*., 2015; Singh and Singh, 1995).

Potato varieties, seed sources and virus distribution. The results showed significant variation ($P<0.05$) in the level of virus infection among the different potato varieties grown across the country (Table 7), with a wide distribution covering over 90% of the growing areas indicating the susceptibility of varieties

grown. The high incidence of virus infection recorded in variety Rwagume, Rwashaki and Kinigi was attributed to the high susceptibility of these varieties (Byarugaba *et al*., 2020), lack of clean seed for these varieties from the national seed potato programme to reach many farmers forcing most of the farmers to use home saved (Own seed) (Aheisibwe *et al*., 2015). Therefore, continuous utilisation of own seed from the susceptible varieties raises the risk of virus infection requiring increased supply and utilisation of clean seed to cab down the risk of virus infection.

The majority of the potato fields were planted with home saved seed (Table 7), which resulted in pre-infected seed further resulting in high virus prevalence levels observed in this study, due to the primary infections as result of using recycled seed that further affected the virus distribution in the country. Davie *et al*. (2017) reported that the practice of seed recycling coupled with vegetative propagation nature of potato crop, presented increased chances for the accumulation of viral diseases causing high virus incidence in the field which lead to seed degeneration and reduction in yield. Most farmers in Uganda were reported by Priegnitz *et al*. (2019) to prefer to plant small sized tubers which are most of the times associated with high load of viruses, perpetuating virus multiplication in the next generation; which also explained the observed high prevalence levels of virus infection in this study.

Incidence and severity of the virus diseases. The high virus disease prevalence and severity observed in mid-western sub-region (Mbarara and Lwengo), Central (Kibaale, Mubende) and Mid northern (Pader) sub region could be attributed to the fact that these districts were on a lower altitude (1088-1334 m.a.s.l), with relatively warmer climates (22.76-26.25 ^{o}C) that favour the aphid (*Myzus persicae*) activity and multiplication; which transmit the viruses in a persistent manner (Hutton *et al.* (2015). This indicates that the low altitude potato growing areas with warmer climates serve as virus pressures zones for potato production in Uganda. The low virus prevalence level in West Nile Sub-region (Zombo) could attributed to the altitude differences and low potato cropping intensity.

In addition, Zombo district being a high altitude area (1800 m.a.s.l), was associated with moderately low temperatures (18-22.3 ^{o}C), could have affected aphid activity in transmitting the viruses in the fields compared to the low altitude areas of mid-western sub-region (Mbarara and Lwengo), Central (Kibaale, Mubende) and Mid Northern sub region that had high temperature (22.76-26.25 ^{o}C). Chung *et al.* (2016) indicated that virus transmission efficiency by vectors was affected by temperatures of 22-25 ^{o}C, that increased aphid activity; and below 22 ^{o}C reduced aphid activities and so the sub region temperature which ranged between 18-22 ^{o}C could have reduced the activities of the vectors.

Kigezi, Elgon and Rwezori sub regions had an average temperature range of 18-21 ^{o}C (18.39, 19.09 and 20.85 ^{o}C); and were on a higher altitude (> 1600 m.asl) which could have impacted on the vector transmission efficiency. Therefore, the prevalence levels of viruses observed in these high altitude sub regions could be associated with the seed recycling behavior of the farmers as most the farmers had used home saved seed.

The low altitude areas of 1274-1344 m.a.s.l contributed 67.4% of the areas identified under moderate risk and 61.4% for high risk which indicated that the low altitude area should be avoided when growing seed potato for susceptible varieties; as these areas serve as virus pressure zones for potato production, and any production in these areas increases the risk of virus infection leading to reduced potato productivity, unless varieties resistant to viruses are to be used. This observation was supported by Steinger *et al*. (2014), who reported that the risk of virus infection decreased with increasing altitude with variety susceptibility influencing the risk of infection.

The risks of virus infection were positively correlated with temperature, but negatively correlated with altitude; suggesting that temperature conditions and altitude affected severity and incidence of virus diseases. This is observation is in line with report by Thomas-Sharma *et al*. (2015) that indicated that potato virus infection was temperature sensitive with increased susceptibility in warmer temperatures than in cooler areas.

CONCLUSION

Six potato viruses (PVS, PVY, PLRV, PVX PVM and PVA) were identified as viruses of agricultural importance in potato production, occurring both as single and mixed infection with wide scale distribution across the potato growing areas in Uganda. The low altitude areas are at high risk of virus infection and, therefore, potato production could be intensified in areas where there is less virus pressure, mainly in parts of West Nile and Rwenzori regions.

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