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DISTRIBUTION AND BIOLOGICAL CHARACTERIZATION OF POTATO VIRUS Y IN KENYA

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ABSTRACT

The aim of this study was to determine the distribution and biologically characterize Potato Virus Y (PVY) infecting potato (*Solanum tuberosum* L.) in Kenya. A survey was done in the main potato growing areas in Kenya. Biological characterization of the PVY Kenyan variants was done by inoculating them into selected indicator plants mechanically and by aptarate aphids *Myzus persicae* and *Macrosiphum euphorbiae*. PVY disease incidence was highest in Imenti Central (91%) followed by Nyandarua North (76%) and Kiambu (73%). The least infection was in Bomet (2%), followed by Narok South district (10%). There was significant mean difference in the ELISA positive samples among the regions of survey (F=10.08, p=0.0001). Meru region had the highest PVY disease infection, although not statistically different from Kiambu, Nyandarua, Uasin Gishu and Mt. Elgon regions. All the indicator plants tested became infected with PVY and expressed varied symptoms except *Datura stramonium* and *Chenopodium murale*. *M. persicae* was a more efficient transmitter of PVY at p=0.037 than *M. euphorbiae*. *S. nigrum*, *Physalis floridana*, *Datura metel*, *Nicotiana glutinosa*, and *Nicotiana tabacum* were the most suitable in detecting PVY. Regions with low PVY incidence should be considered as sites for seed multiplication and continuous surveillance for PVY should be done so as to understand the spread pattern in Kenya

Keywords: *Incidence, Myzus persicae, Macrosiphum euphorbiae, potato, Survey.*

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INTRODUCTION

Potato is the second most important food crop in Kenya after maize (Ministry of Agriculture, 2007). The crop is one of the most important sources of income and employment in the rural areas (Olanya, 2006). The annual potato acreage in Kenya is about 100,000 hectares, which is distributed among approximately 500,000 smallholder farmers. The average annual production is about 1 million tonnes with an average yield of 7.3 metric tonnes per hectare. Potato is mainly cultivated in the high altitude areas (1500-3000m above sea level) (Obare ., 2010). These areas include Meru, Embu, Kirinyaga, Laikipia, Nyeri, Muranga, Kiambu and Nyandarua Mau Narok, Molo), Tinderet, Nandi Escarpment and Cherangani hills (Kirumba , 2004). According to Nyamongo (2009), the national production is far below the potential, largely due to limited use of certified seeds. There is inadequate supply of certified seeds to the extent that farmers depend on informal seed sources (farm-saved, local markets or neighbours) According International Potato Center (CIP) (2008), viral diseases especially PVY cause yield losses of up to 2.8 tonnes per hectare and these viral diseases pose a serious constraint to potato production particularly PVY. Recycling of virus infected potato seed leads to build up of potato viruses, including PVY, and other diseases. In Kenya, only

4% of the farmers have access to clean seed (Kaguongo ., 2008). Therefore viral diseases are expected to be high among potato farmers but there is no clear report on the status of potato viruses in potato growing areas of Kenya.

The transmission of PVY to potato plants is through grafting (stem- and tuber-grafting), plant sap inoculation and aphid transmission. PVY has been reported to spread by plant-to-plant contact, for instance in tobacco and tomato crops in Southern America (Whitworth ., 2010). It can also be transmitted by contact between sprouts of potato tubers during storage. Transmission by seed has been reported in *S. nigrum* and *Nicandra physaloides* (Kerlan and Moury, 2008). The most common manner of PVY transmission to potatoes in the field is by aphids (Halbert, 2003). Hosts such as weeds and other crops serve as breeding grounds for these aphids and form a temporary area of colonization before the aphids migrate to the potato fields (Radcliffe, 2002).

The green peach aphid (*Myzus persicae*) has been found to be most effective in its role as a viral vector (Kanavaki ., 2006), but others such as *Aphis fabae*, *Aphis gossypii*, *Aphis nasturtii*, *Macrosiphum euphorbiae*, and *Rhopalosiphum insertum* are also associated with viral transmission (Halbert, 2003). *M. euphorbiae* is the most abundant aphid in Kenya (Were ., 2003). Aphids can either be colonizing or non-colonizing; colonizing aphids reproduce and establish themselves on potato plants, while non-colonizing aphids do not reproduce nor establish colonies on potato plants. Transmission of PVY by aphids occurs in a non-persistent, non-circulative manner which suggests a less intimate interaction between virion and vector than is the case of circulative virions (Gray, 1996). The viral replication does not occur within the aphid vector and that, unless the aphid feeds on infected plants, it loses its ability to infect plants after two to three feedings. The virions attach to the aphid stylet within seconds and remain infectious for 4 to 17 hours. The distance over which the virions can be transmitted is limited due to the short period for which they remain infectious (Robert, 2000).

PVY virions consist of non-enveloped filamentous structures that are 680 - 900nm in length and 11 to 15nm in width (Urcuqui . (2001); Edwardson, 1947). Morphologically the potyvirus consists of approximately 2000 copies of coat protein (CP) which forms a cylindrical inclusion body (Cib). The Cib encapsulates a single strand of positive sense RNA which is in the order of 10kb in length and has a non translated 5'-terminal region (5'-NTR) as well as a 3'-poly-A tail (Edwardson, 1947). Serological detection of PVY involves the use of antibodies specific to a strain or a group of strains of the virus. Enzyme-linked immunosorbent assay (ELISA) has been the most common serological technique used in plant virus detection (Albrechtsen, 2006).

PVY has different isolates (PVY^C, PVY^N and PVY^O) depending on the symptoms they induce in various potato plant species. In potato crops, the PVY^O strain occurs worldwide. The PVY^N strain has been recorded in South America, Europe, Africa, Asia and New Zealand whereas it is a quarantine pathogen in Canada and USA, with localized outbreaks (Croslin ., 2002). The virus has also been reported to infect potato crop and potato seed in stores in Kenya (Olubayo ., 2011; Were ., 2014)). No work had been done to ascertain the distribution and biological characterization of PVY in potato growing regions of Kenya.

MATERIALS AND METHODS

Survey for PVY

A survey was done during the long rain growing season in June 2011 in Eastern, Central, Rift Valley and Western regions of Kenya. A GPS device (Triton 'windows CE core 5.0' X11-15302) was used to record the coordinates and altitude of the sample location. Visual symptoms of mosaic, leaf rolling, dwarfing, chlorosis, or a combination of these were assessed just before flowering. Each field was divided into quarters, disease incidence was assessed and defined as the number of infected plants in each plot expressed as a percentage of total number of plants observed. This was done by selecting one site per quarter of 5m by 30m to give a representative figure. Leaf samples from potato plants with PVY symptoms were randomly sampled into sample bags for analysis.

Serological detection of PVY

The Monoclonal antibody (Mab) DAS-ELISA reagent set for PVY^{O/C/N} (SASA, Scotland, UK) was used according to the manufacturer's instructions to detect PVY in all the field potato plants showing PVY-like symptoms. Samples (1 cm in diameter) were ground in 5 in 500 ml extraction buffer (50 mM sodium phosphate containing 20 mM sodium sulphite) in a 1.5 ml in a plastic bag before being tested by DAS-ELISA.

Transmission of the Kenyan PVY isolates

Seeds of selected indicator plants (*Nicotiana benthamiana*, *Datura stramonium*, *Physalis floridana*, *N. glutinosa*, *Chenopodium amaranticola*, *Chenopodium quinoa*, *Solanum lycopersicum* L. *N. tabacum*, *Chenopodium murale* and *Datura metel*) were planted in sterilized soils in a screen house and transplanted in polythene pots. To improve uniformity of germination, the seeds were dried for 24 h before planting and transplanted after three weeks.

Infected leaf tissues were ground in inoculation buffer (50mM Na₂HPO₄ 12 H₂O; 50mM KH₂PO₄, 0.44% (w/v) sodium diethyldithiocarbamate; pH 7.4) using a mortar and pestle. Carborundum powder was dusted onto leaves of indicator plants before the mixture was rubbed onto indicator plants using cotton wool swab soaked in the mixture by starting each stroke from the petiole, and ending at the leaf tip. A "control set" of indicator plants was inoculated with pure buffer as described by Lacroix . (2010).

Aphid samples were obtained from the University of Nairobi, Department of Plant Science and Crop Protection. They were reared on healthy 4-week old cabbage plants in an insect-proof mesh cage (90 by 60 by 60 cm wooden frames). Insects parasitizing on aphids may be controlled by specific insecticides. Colonies were checked regularly for parasite appearance. The colony was renewed whenever the black growth of saprophytic fungi on the surface of leaves is was noted. Mites that induced aphid population decline were controlled by disinfecting the cages with a miticide (Cyclone®) as described by Fajinmi and Odebode, (2011).

The reared aphids of *M. persicae* and *M. euphorbiae* used as virus vectors, were starved for 2 h, and kept in wide test tubes (6-cm long and 2-cm) protected with musclin-cloth to ensure free flow of air. Aphids were gently picked by a camel hair brush and placed onto PVY infected *N. tabacum* cv. Samsunplants. They were allowed an acquisition access period (AAP) of 5 min to acquire the virusof feeding. Viruliferous aphids were then transferred to healthy indicator plants (5aphids per plant) with an inoculation access period (IAP) of 30 min for feeding while healthy plants were exposed to non-viruliferous aphids as controls before they were sprayed with an insecticide (Cyclone®). The inoculated indicator plants were maintained in the screen house for symptom development and observation as described by Volkov . (2009). All the test plants were subjected to ELISA tests to confirm presence of PVY and the proportion of plants infected were recorded.

RESULTS AND DISCUSSION

RESULTS

Potato virus Y incidence

The mean PVY disease incidence was obtained per the surveyed district. During the survey 493 leaf samples showing viral symptoms from farmer’s fields were collected and taken to the laboratory for analysis using DAS-ELISA. The results were as shown in Table 1.

As shown in Table 1, there were very high PVY incidences in Central Imenti, North Imenti and Nyandarua North. High PVY incidences were in South Imenti, Nyandarua Central, Nyandarua West and Kiambu. Low PVY incidences were in Narok South, Bomet Mt. Elgon and West Pokot. There were 0% PVY incidences in Narok North, Molo, Uasin Gishu, Keiyo North, Marakwet West and Trans Nzoia. Among the 493 samples that were subjected to DAS ELISA, 133 tested positive for PVY.

There was significant mean difference in the ELISA positive samples among the regions of survey (F=10.08, p=0.0001). Meru region had the highest PVY disease infection, although not statistically different from Kiambu, Nyandarua, Uasin Gishu and Mt. Elgon regions. The least PVY disease infection was at Narok region but not statistically different from Molo and Bomet regions (Table 2).

Table 1. Virus disease incidence per district of survey and ELISA results

District	Average altitude (masl)	Visual Virus disease incidence (%)	ELISA		N
			No. of PVY Positive samples	% of total	
S. Imenti	2152.50	4.05	20	66.7	30
N. Imenti	2291.42	13.49	16	76.2	21
C. Imenti	2394.85	66.19	19	90.5	21
Nyandarua N.	2481.50	4.78	15	75.0	20
Nyandarua C.	2289	93.08	15	68.2	22
Nyandarua S.	2456.80	66.91	8	40.0	20
Nyandarua W.	2678.28	46.33	8	72.7	11
Kiambu	2167.28	21.56	22	73.3	30
Narok S.	2355	40.27	1	10.0	10
Narok N.	2394	46.43	0	0.0	30
Molo	2600.25	22.48	0	0.0	70
Bomet	1859	8.46	1	2.0	53
Uasin Gishu	2140	30.34	0	0.0	30
Keiyo N.	2082	61.84	0	0.0	20
Marakwet W.	2580	44.86	0	0.0	35
Trans-Nzoia	1860	21.60	0	0.0	10
Mt. Elgon	2100	28.00	5	12.5	40
West Pokot	2182	60.05	3	15.0	20
Total			133	27.0	493

N = Total number of samples

Table 2. Mean PVY disease incidence of regions surveyed

Region	N	Mean virus incidence	Region/ Blocks
A	6	4.3454	A - Meru
A	3	4.2646	C - Kiambu
A	7	4.0477	B - Nyandarua
* B A	1	3.4012	G - Uasin Gishu
* B A	2	3.1985	H - Mt. Elgon
B	1	2.3026	F - Bomet
B	1	2.3026	E - Molo
B	1	2.3026	D - Narok

* Means with different letters are significantly different. (p=0.0001, r=0.05). N= number of districts per region of survey

PVY indicator plant interaction

Mechanical transmission

PVY from potato induced leaf mottling, veinal necrosis and leaf distortion on 30% of *N. tabacum* cv White Burley fifteen days after inoculation. Local lesions were induced on *Chenopodium amaranticolor*. On *Physalis floridana* PVY induced inter-veinal chlorosis (Fig. 1 Table 3), collapse and premature death of this plant while on *Datura metel* the virus induced vein clearing without any leaf distortion after fifteen days of inoculation. PVY induced mild mosaic and yellowing of the leaves on *Solanum lycopersicon*. It caused stunting, mild mottling and chlorotic necroses on *Chenopodium quinoa* (Fig. 1).

Table 3. PVY virulence on indicator plants after mechanical inoculation

Indicator plant	N	Visual positive	ELISA Positive	% of total
<i>Nicotiana tobacum</i>	10	5	3	30
<i>Physalis floridana</i>	5	4	4	80
<i>Datura metel</i>	7	2	0	0
<i>Solanum lycopersicum</i> L.	5	2	0	0
<i>Solanum tuberosum</i>	5	3	2	40

N= Number of test plants inoculated



Figure 1. PVY symptoms observed on indicator plants and potato after mechanical and vector transmission

(a)veinal necrosis and leaf distortion on *Nicotiana tabacum*, (b)Mosaic, leaf deformation on *Nicotiana glutinosa*, (c)interveinal chlorosis on *Physalisfloridana*,(d)Mosaic patterns on *Solanum tuberosum*, (e)mottling on *Chenopodium quinoa*, and (f) mosaic and leaf deformation on *Solanum nigrum*.

Solanum nigrum, *Physalis floridana*, *Datura metel*, *N. glutinosa*, and *N. tabacum* "White Burley" were the most suitable in detecting PVY as they showed the most distinct symptoms. *Chenopodium quinoa*, *Chenopodium amaranticolor* and *Solanum lycopersicum* L. "Rutgers" and *Solanum incunum* showed mild symptoms that were less distinct (Table 4).

Table 4. Suitability of indicator plants for PVY detection

Indicator plant	Transplanted (days)	Inoculated (days)	Symptom	Suitability for PVY detection
Solanum nigrum	28	46	Mosaic, leaf deformation	++
Solanum incunum	28	46	Mottling, stunting	+
Nicotiana glutinosa	28	46	Mosaic, leaf deformation	++
Nicotiana tabacum	28	46	Mosaic, stunting	++
Chenopodium amaranticolor	28	46	Chlorotic spots	+
Physalis floridana	17	31	Stunting, mosaic	++
Datura metel	17	31	Mottling, vein clearing	++
Datura stramonium	17	31	No symptoms	-
Chenopodium quinoa	17	31	Chlorotic spots	+
Chenopodium murale	17	31	No symptoms	-
Solanum lycopersicum	17	31	Yellowing of leaves, mosaic	-

*++, suitable, all inoculated plants showed symptoms;
 +, not suitable, only some of the inoculated plants showed symptoms;
 -, not suitable, none of the inoculated plants showed symptoms

Datura stramonium and Chenopodium murale were the least suitable for detecting PVY as they did not show any foliar symptoms and also indicated negative ELISA test. Datura stramonium and Solanum lycopersicum L. tested negative for PVY after inoculation despite showing mild visual symptoms.

Transmission of PVY by aphids

The aphids (*M. persicae* and *M. euphorbiae*) transmitted PVY to indicator plants and produced distinct symptoms. *Physalis floridana*, and *Solanum tuberosum* recorded the highest proportion of plants transmitted with PVY by *M. persicae*. *Nicotiana tabacum* had the highest proportion of plants transmitted with the virus by *M. euphorbiae* followed by *S. tuberosum* and *C. amaranticolor* while *Chenopodium murale* and *Datura stramonium* were not effectively transmitted with the virus (Table 5).

Table 5. PVY virulence on indicator plants after Aphid transmission

Aphid vector	Indicator plant	No. of Plants inoculated(N)	Number positive	Proportion
<i>M. persicae</i>	<i>C. amaranticolor</i>	4	2	50
	<i>C. murale</i>	7	0	0
	<i>D. stramonium</i>	9	0	0
	<i>L. esculentum</i>	5	2	40
	<i>N. glutinosa</i>	8	3	37.5
	<i>N. tabacum</i>	6	3	50
	<i>P. floridana</i>	3	2	66.67
	<i>S. incanum</i>	2	1	50
	<i>S. nigrum</i>	4	1	25
	<i>S. tuberosum</i>	3	2	66.67
	<i>M. euphorbiae</i>	<i>Ch. amaranticolor</i>	5	3
<i>Ch. Murale</i>		5	0	0
<i>D. stramonium</i>		4	0	0
<i>L. esculentum</i>		5	2	40
<i>N. glutinosa</i>		5	4	80
<i>N. tabacum</i>		6	4	66.67
<i>P. floridana</i>		5	2	40
<i>S. incanum</i>		6	3	50
<i>S. nigrum</i>		4	2	50
<i>S. tuberosum</i>		5	3	60

There was significant difference in PVY transmission efficiency from infected potato plants to indicator plants between *Myzus persicae* and *Macrosiphum euphorbiae* ($F=5.06, p=0.0372$). *Myzus persicae* had a higher PVY transmission efficiency than *Macrosiphum euphorbiae* (Table 6).

Table 6. Aphid transmission efficiency of *Myzus persicae* and *Macrosiphum eurphobiae*

Aphid	N	Mean virus infection	Std. Dev	Std. Error
<i>Myzus persicae</i>	10	70.67	22.87	7.23
<i>Macrosiphum euphorbiae</i>	10	50.65	15.83	5.01
Total	20			

N= number of indicator plants transmitted with the virus

DISCUSSION

PVY disease incidence was high in Meru and Kiambu regions which also recorded 2241.83m asl. The region receives an average rainfall and relatively high temperatures which are ideal conditions for aphid activity that plays a key role in virus transmission. When potatoes are out of season, aphids survive on alternative hosts as farmers do not weed the volunteer crops which help in spreading the virus (Muthomi ., 2011). Since PVY symptoms are not as clear as the other diseases, most farmers tend to ignore or do nothing about them. This could explain the high virus incidence. Potyviruses can occur in synergistic infections and PVY is known for the diversity it displays whenever it occurs. The widespread occurrence of PVY in Kenya may result in synergistic association with other plant viruses or the formation of recombinant strains that may have devastating consequences on the production of potato crops. The high prevalence and incidence of PVY in Kenya require an effective disease management strategy. Management of PVY can be achieved by controlling its vector. However, chemical control of the vector (aphids) is difficult due to their high mobility and the high speed of virus transmission as the aphids acquire the virus non-persistently. Another alternative control measure is the use of resistant cultivars but the sources of resistant genes to the virus are not common and in cases in which they are available, they are easily overcome by the emergence of new strains of the virus due to virus recombination.

Biological assays require artificial transmission of the test virus onto different host or indicator plants. This was achieved by mechanical transmission as illustrated by Albrechtsen (2006). Some indicator plants show visual symptoms but tested negative for PVY. These symptoms could have been caused by other physiological factors in the screen house or stress injuries caused by caborendum powder spray during mechanical inoculation other than viral infection. PVY was successfully detected in some of the indicator plants tested with clear symptoms (*N. benthamiana*, *Physalis floridana*, *N. glutinosa*, *Chenopodium amaranticola*, *Chenopodium quinoa*, *Nicotiana tabacum*, *Datura metel*) but not in *Datura stramonium* and *Chenopodium murale*. These findings agree with those of Verhoeven and Roenhorst (2003) that this biological assay provides the broadest detection method for potato-infecting viruses. PVY induced veinal necrosis and vein clearing in *N. tabacum* plants which was in agreement with earlier findings by Verhoeven and Roenhorst (2003). These varied symptoms indicated that there were mixed strain infections of PVY. This finding revealed the diversity of PVY strains infecting potato in Kenya as also reported in other parts of the world by Kerlan and Moury (2008). In *Nicotiana glutinosa*, the virus induced mild leaf mosaic, crinkling, systemic necrosis leaf drop, streak and dwarfing which was in agreement with other findings by Schubert . (2007).

When aphids fed on PVY infected *Nicotiana tabacum* plants, a period of 15 minutes was enough to transform non viruliferous aphids to viruliferous ones. Aphids acquired the virus for a brief period of feeding on the epidermal tissues of infected plants and then the aphids transmitted the virus immediately to indicator plants. DAS ELISA conducted on transmitted leaves and the non transmitted top leaves 7-14 days after inoculation confirmed the systemic infections with PVY in top leaves. *Nicotiana tabacum*, *Nicotiana glutinosa*, and *Datura metel* can be used as diagnostic species susceptible to all PVY strains.

The results of the survey show that PVY is one of the most economic important pathogens of potato in Kenya. This agrees well with the general notion that potyviruses are very important viruses. Additional molecular or serological tests are required for final identification of the viruses detected. Regions with low PVY incidence should be considered as possible sites for seed multiplication.

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