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Review

Next generation sequencing platforms for potato virus hunting, surveillance and discovery

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Potato (*Solanum tuberosum* L.) is a key alternative to maize crop in Kenya. However, pests and diseases affect the yields. Information on Irish potato virology is continually patchy. Viral disease dynamics require constant updating to track new and novel agents. Efforts to mitigate viruses and crop breeding for tolerance can be determined this way. In Kenya, key potato viruses include: *Potato Leaf Roll Virus* (PLRV), *Potato Virus X* (PVX), *Potato Virus S* (PVS) and *Potato Virus Y* (PVY). Detection of these viruses has been through symptomatology, serology and nucleic-acid approaches. Molecular biology has revolutionary developments in sequencing technologies influencing diagnosis of plant viruses. Massive parallel sequencing has promoted detection, identification and discovery of novel viruses in plants without use of antibodies or prior virus knowledge. Complete viral genomes can be sequenced from asymptomatic and symptomatic samples. Viral metagenomics, diversity and genome variability can be deduced this way. Next generation sequencing platforms bring robustness, timeliness and affordability to virus detection. However, few studies have attempted to utilize it in unravelling potato virology beyond the routine detectable agents in the country. The current study reviews diagnosis of Irish potato viruses in Kenya against the techniques used, comparing them to next generation sequencing.

Key words: Deep sequencing, next generation sequencing, reverse transcriptase polymerase chain reaction (RT-PCR), serology.

INTRODUCTION

Potato is ranked the fourth most important food crop globally, with a production of 388 million tons in 2017, after rice (770 million tons), wheat (771 million tons) and maize (1.1 billion tons). It is the third most important food crop since maize as a food crop is at 14% (FAOSTAT, 2019). The IPBO (2019) documents that; Africa potato production has increased. Kenya is among the top 6 leading producers alongside Algeria, Egypt, South Africa and Morocco. Pests and disease production constraints have been documented (CIP, 2019; FAOSTATS, 2019).

Viruses contribute to over 47% of the total plant emerging infectious diseases (Anderson et al., 2004).

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These viruses are widespread and threaten as emerging crop virus infections (Craig et al*.,* 2004). Potato viruses contribute to reductions in quality and quantity of potato in Sub Saharan Africa (SSA). Losses due to the viruses have been experienced in many SSA countries. For instance; *Potato Virus Y* has devastated production in Kenya, Uganda, Ethiopia and South Africa; *Potato Leaf Roll Virus* and *Potato Virus X* (havocked Kenya and Uganda); while *Potato Virus A* damaged production in Kenya (Gildemacher et al. 2009; Ibaba and Gubba, 2011; Wangai and Lelgut, 2013). The Kenyan Irish potato tonnage has had an unsteady precedence between the years 2000 and 2010 (FAOSTATS, 2011). Potato viral infections manifest symptoms in some cases while other viruses have total asymptomatic characteristics. Visual diagnosis seldom differentiates one viral infection from the other. Among potato viral diseases, stunting, necrosis, mosaic, and leaf roll are most important and caused by viruses such as *Potato Virus X*, *Potato Virus M* (PVM), *Potato Virus S* (PVS), PVY, *Potato Virus A* (PVA), *Potato aucuba virus, potato leaf roll virus* (PLRV), *mop-top virus* and *Potato apical leaf roll virus* (APLRV) (Awasthi and Verma, 2017).

Experimental virus indexing has documented high prevalence of potato viruses (Gildemacher, 2012; Machangi et al*.,* 2004; Olubayo et al., 2010). The viruses responsible for majority of yield reductions are *Potato leaf roll virus* (PLRV), *Potato virus Y* (PVY), and *Potato virus X* (PVX) that occur in combination with mild viruses like *Potato virus A* (PVA), *Potato virus M* (PVM) and *Potato virus S* (PVS) (Kabira et al., 2006; Schulte-Geldermann et al., 2012). PVY is the most important virus globally (Lacomme et al*.,* 2017), despite *Potato Leaf Roll Virus* being considered the most economically potent virus. However, studies have shown that *Potato virus Y* and *Potato Leaf Roll Virus* are the most significant viruses infecting South African potatoes (Denner et al., 2012).

Characterization of these viruses has been based on biological assay, microarray, electron microscopy, nucleic acid based techniques like PCR and serological techniques such as enzyme linked immunosorbent assay (ELISA) (Boonham et al*.,* 2008; Ng et al*.,* 2011). Any investigation of viral dynamics in wild plant species needs clear background of plant biochemical and structural features. ELISA and Reverse Transcriptase-Polymerase Chain Reaction for a phloem-restricted nature study model system (plant virus BYDV-PAV) makes the findings relevant to detection sensitivity in plant-microorganism systems. The methodological approaches tested show importance of optimizing and assessing virus detection techniques for application in wild plant hosts. Such information is critically needed (Lacroix et al*.,* 2016). This is consistent with similar studies (Kunta et al*.,* 2014; Pereira and Lister 1989; Rashed et al*.,* 2014; Sanchez-Navarro et al*.,* 2007) in other plants that have not been well exploited.

In plant screening, biological assay and microscopy are ancient (Matthews, 1991). This technique is dependent on high quality indicator and propagative host plants. Serology and PCR rely on known agents only (Coetzee et al., 2010; Yanagisawa et al., 2016; Zheng et al., 2017). Other than over-reliance on high quality indicators, test results are subjective and not confirmative in Electron microscopy. Introduction of serological assay (ELISA) used antibodies for detection of viruses (Clark and Adams, 1977), followed by PCR clonal amplification of DNA (Candresse et al*.,* 1998). ELISA is cost effective, robust and amenable (Boonham et al*.,* 2014); however, it has restricted use to universally known agents and is unable to detect novelty especially in viroids (Grothaus et al., 2006). Molecular diagnostics advantages include high turnover rate, ability to identify individual strains and scalability to high throughput (Martin, 2012). Viral RNA is reverse transcribed from RNA+/- strands by the RT enzyme to synthesize cDNA (Ali et al., 2014).

Whereas molecular techniques are highly sensitive and specific than serology, symptomatology is error prone due to an overlap in the manifest symptoms (Notomi et al*.,* 2000). *Potato Leaf Roll Virus* is asymptomatic especially late infection of potato by the *SymlessLS10 Potato Leaf Roll Virus* isolate (Hühnlein et al., 2016). Unlike culture methods, real time PCR (qPCR) has demonstrated high reproducibility and less variability (Dong et al., 2016). This is consistent with similar studies by Hockman et al. (2017) that merit reverse transcriptase PCR (RT-PCR). However, these techniques are culpable of significant drawbacks in detecting unknown viral agents either in a new host or novel agents due to sole reliance on routine agents by sequence or antibodies. Advent of novel technologies such as Next generation sequencing has been used in diagnosis and characterization of new viruses affecting various plants (Prabha et al*.,* 2013).

CHARACTERIZATION OF POTATO VIRUSES IN AFRICA

Potato viruses have colonized numerous potato growing nations across Africa. Detection of the virus and viroids has utilized varied diagnostics in documenting incidence, prevalence and occurrence as shown in Table 1. In North Western Cameroon, studies exploring prevalence of six potato viruses applied Double Antibody Sandwich-ELISA (DAS-ELISA) to confirm six viruses (*Potato Virus A, Potato Leaf Roll Virus, Potato Virus M, Potato Virus S, Potato Virus X and Potato Virus Y*) as prevalent in the country (Njukeng et al., 2013). Less sensitive versions of ELISA, {Multi-array test strips (MALTS)}, have viral antibodies that were used to detect eight viruses in potato, but are less sensitive (Safenkova et al*.,* 2016).

Similar studies investigating the incidence of *Potato Virus X, Potato Virus S, Potato Mop Top Virus, Potato Virus M, Potato Virus A, Tomato Spindle Wilt Virus* and *Potato Spindle Tube Viroid* in South Africa applied

Table 1. Potato virus diagnostic tools and the African country where used. South Africa is the most consistent in use of the majority of tools.

Potato virus diagnostic tool	African country applied	Reference
Serology (i) DAS ELISA (ii) MALTS ELISA (iii) NCM ELISA	Continental (Cameroon, Tanzania, Kenya, S. Africa, Tunisia, Sudan…)	Njukeng et al. (2013), Safenkova et al. (2016), Lezan (2017), Wiets (2013), Evangelista (2013), Baldo et al. (2010), Bondole (1992) Were et al. (2013), Muthoni et al. (2009), Okeyo (2017), Nyamwamu et al. (2014), Nyaboga et al. (2008), Larbi et al. (2012)
Molecular (i) RT-PCR (ii) RT-qPCR (iii) PCR	South Africa, Tanzania,	Wiets (2013), Botermans et al. (2013), Boonham et al. (2004), Verhoeven et al. (2004), Bostan et al. (2004), Shamloul et al. (1997), Lezan (2017), Wiets (2013), Espach (2015), Kumar et al. (2017), Zhang et al. (2017), Evangelista, (2013)
NGS (i) Roche 454 /Illumina/SOLiD/Ion Torrent	South Africa	Lezan (2017)

reverse transcriptase polymerase chain reaction (RT-PCR) CP-gene amplification and whole genome amplification for PSTVd and detected only two viruses as present in the samples (*Potato Virus S* and *Potato Virus X*); while *Potato Leaf Roll Virus* was reported to have attained a reduced pathogenicity (Wiets, 2013). The study suggests that the other viruses failed to be detected by RT-PCR either due to failure of amplification or absence of the agents (Wiets, 2013). Potato Spindle Tuber Viroid (PSTVd) is distributed widely around the globe (CABI/EPPO, 2014). Primers have been designed specifically for pospiviroid amplicon generation in Real-time PCR or conventional PCR that is used for successful detection of PSTVd (Boonham et al*.,* 2004; Bostan et al., 2004; Botermans et al*.,* 2013; Shamloul et al*.,* 1997; Verhoeven et al., 2004). Studies by Lezan, (2017) and Wiets (2013) are consistent in the use of ELISA as the routine testing tool by the South African Seed Potato Certification Scheme, for viruses such as PLRV. Though RT-PCR assay is amenable for epidemiological studies and certification schemes to detect *Potato Leaf Roll Virus* early in potato crops (Hossain et al*.,* 2013), post agarose gel electrophoresis analysis is time consuming and less accurate.

RT-PCR and Next Generation Sequencing have been used in validating better tools for potato certification of *Potato Leaf Roll Virus* and identification of coding regions in potato viruses in the Sandveld region South Africa. Through the study, noncoding 5' and 3' regions of the genome were compared using Next Generation Sequencing other than identification of novel potato viruses, leading to endorsement of RT-PCR and Next Generation Sequencing as better tools in characterizing potato viruses (Lezan, 2017). Furthermore, the same study used Next Generation Sequencing (Ion Torrent Sequencing) to gain more information on *Potato Leaf Roll Virus* prior to tracing its ancestry in relevance to global strains. Similarly, RT-qPCR has been developed in South Africa for the detection of *Potato Leaf Roll Virus* in potato leaves and tubers (Espach, 2015), though it is comparatively expensive than ordinary RT-PCR (Coudray-Meunier et al*.,* 2016). In studies to detect *Potato Spindle-Tube Viroid*, *Potato Virus A, Potato Virus S, Potato Virus X, Potato Virus Y, Potato Leaf Roll Virus* and *Potato Virus M*, Multiplex PCR has been successfully adopted (Kumar et al., 2017; Zhang et al*.,* 2017).

In Central Tunisia, the presence of the six most economically important viruses: *Potato Leaf Roll Virus, Potato Virus S, Potato Virus M, Potato Virus X, Potato Virus A* and *Potato Virus Y* were determined across various incidence levels. Serological tests ranged from 0.5% (*Potato Virus M*) to 71% (*Potato Virus Y*). The variability of *Potato Virus Y* was analyzed by a combination of serotyping, indexing on tobacco and RT-PCR tests of the 2 genomic regions (5′NTR/P1 and CP/3′NTR). Serological samples revealed dominance of the PVY^{N} strain (88.2% of total PVY positives). Furthermore, strains subjected to molecular typing revealed that 73.3% of the PVY^{N} strains were PVY^{NTN} variants having a recombination junction at the CP/3′NTR region across 94.4% of their totals, though no recombination junction was found in the genome of the isolates of PVY^{N} group (Larbi et al*.,* 2012).

In Eastern Africa, focus has been on the use of DAS-ELISA, NCM-ELISA and RT-PCR in the diagnosis of potato viruses. DAS-ELISA was adopted in Mbeya region of Tanzania to determine whether *Potato leaf roll virus* (PLRV), *Potato virus S* (PVS), *Potato virus A* (PVA), *Potato virus Y* (PVY), *Potato virus X* (PVX) and *Potato virus M* (PVM) are present in potato. Though DAS-ELISA confirmed the presence of all the six viruses, further analysis using RT-PCR dismissed occurrence of PLRV PVS and PVY (Evangelista, 2013.) Here, occurrence of PLRV, PVA, PVM, PVS, PVY and PVX was determined from 219 potato accessions in Mbeya regions of Kawetele, Umalia, Uyole, Kikondo, and Rungwe

(Mwakaleli) in Tanzania. Virus-like symptomatology was observed in most fields, including: leaf rolling, yellowish-green mosaic, and vein necrosis. The ten symptomatic and three asymptomatic leaves sampled from each field and tested by double antibody sandwich (DAS)-ELISA tested positive for PVS and PLRV in 55 and 39% of total samples, respectively. PVM and PVX were positive in 14 and 5% of most fields respectively. Co-infections of PLRV and PVS were detected in 14% of the samples. PVY and PVA were present in two localities. Mixed infections (3 or 4 viruses) were present in 5% of the crops. Twenty samples, from Mwakeleli and Uyole ELISA-positive more than one virus, were analysed using RT-PCR with virus-specific primers for amplifying the coat protein (CP) encoding gene. ELISA-positive leaf samples were subjected and tested positive for RT-PCR. ELISA-negative for the viruses PVA or PVX, were positive when tested by RT-PCR, indicating suitability in actual incidence of the viruses as opposed to DAS-ELISA. The PCR products 5 samples for each virus sequenced, reconfirmed presence of PVA, PVS, PLRV, PVX and PVM.

The incidence of *Potato leafroll virus* (PLRV), *Alfalfa mosaic virus* (AMV) and *Potato virus Y* (PVY) in potato crops was assessed visually and confirmed through direct tissue blot immunoassay at three locations, Elnaiya, Shambat and Elshehinab in Khartoum State, the main potato growing region in Sudan (Baldo et al*.,* 2010). CABI/EPPO 2019 reports (https://www.cabi.org/ISC/datasheet/43762) are keen in reporting viral densities from across the world. Using researcher inferences by varied characterization methods, the reports in Africa, for instance, shows *Potato virus Y* (PVY) densities cladding the continent but limited in Kenya. The report distribution map (https_www.cabi.org_isc_distribution_map.png) also documents historical research into the PVY potato mottling virus across the globe with similar elucidations on incidence, prevalence and distribution.

SEROLOGICAL DETECTION OF POTATO VIRUSES IN KENYA

Studies have always emphasized on the use of immunological or serological diagnosis of crop viruses in Kenya. Initial studies on *Potato virus Y* (PVY) to cross match disease incidence to host range and to compare results applied both ELISA and Electron Microscopy have been done (Bondole, 1992). The study documented prevalence of PVY as high, setting a standard for the use of the two tools in potato pathology. Studies by Were et al. (2013) in Kenyan highlands used ELISA to detect *Potato virus S* (PVS) (dominant virus), *Potato Virus Y* and *Potato Virus X* in potato. Furthermore, the study reported the same viruses in *Solanum nigrum* using DAS-ELISA while PLRV, PVM, PVS and PVY were detected in *Solanum incunum* weed species. The virus strains $PVY^{O,}$ PVY^{N}, recombinant strains PVY^{N-Wi} and PVY^{NTN} were also distinguished using ELISA. Additionally, Muthomi et al. (2009) document using serology to detect PLRV, PVS and PVY strains in potato. Okeyo, (2017) used CIP DAS-ELISA to detect 4/6 viruses (PVS, PVY, PLRV and PVM) in Irish potato in Kenya during his study of resistance in potato genotypes. No single study has gone further to use Next Generation Sequencing in confirming the incidence, prevalence and distribution of the viruses alongside Serology.

Monoclonal antibody DAS-ELISA has been used to confirm presence of *Potato Virus Y* strains in potato, in Eastern, Western, Central and the Rift valley regions of Kenya (Nyamwamu et al*.,* 2014). Similar studies by Muthomi et al. (2011) and Were et al. (2014) used Serology to successfully document strains of PVY in Kenya. Also, Nyaboga et al. (2008) used NCM-ELISA to document key potato viruses including *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic stunt virus* (SPCSV), and *Sweet potato chlorotic fleck virus* (SPCFV). Four PVY strains: (PVY^{O} {common}, PVY^{C} {Stipple streak strain}, PVY^{N} {Tobacco venial necrosis strain} and PVY^{NTN} have also been documented using serology and molecular techniques (John et al., 2013).

Symptomatology has been used as the primary diagnostic tool for viral infections in potatoes. However, the symptoms are highly erratic due to an overlap in manifestation. A number of viruses are equally asymptomatic on infection, implying a great bias in using the tool. In plants, detecting disease agents by symptomatological methods, is less selective and requires intensive expertise to cross-match specific symptoms to an agent (Prabha et al., 2013). Studies using molecular diagnostic tools in potato viruses are scanty. A few publications have attempted to use the tool to portray viral ecogenomics and diversity in the Kenyan case.

The directorate of diagnostics and research laboratory, foundation of Plant services (UCDAVIS-FPS) determines that serology and nucleic acid based technologies are considered expensive per sample (Al Rwahnih et al*.,* 2015), unlike modern diagnostics of plant viruses. This is consistent with individual sample analysis studies that are characteristic in immunological and some molecular based diagnostic tools such as qPCR, RT-PCR, RFLP, Multiplex PCR, Nested PCR and other variants (Marina et al*.,* 2014).

SUCCESS OF NGS IN VIRUS HUNTING AND DISCOVERY

Sequencing uses Capillary Electrophoresis (CE) principles to unravel the genetic code. Throughput, scalability, robustness and speed dictate the various generations of sequencing, with NGS as the most powerful (Capobianchi et al., 2013). Sangers sequencing

is one of the two first generation sequencing platforms that have been used for long to sequence of between 600 and 1000 base pairs (bp) per run, though it is comparatively costly and time consuming (Wu et al., 2015). Sequencing through short read approaches is divided into sequencing by ligation (SBL) and sequencing by synthesis (SBS) (Goodwin et al., 2016; Myllykangas et al., 2012).

The first High Throughput Sequencing platforms (under Second generation Sequencing) was Roche 454 FLX Pyrosequencing platform by 454 life sciences (http://www.454.com). Roche/454 sequencing had an initial market appearance in 2005. The method uses Sequencing by Synthesis (SBS) approach, known as pyrosequencing technique, that relies on the detection of a pyrophosphate released after each nucleotide is incorporated into the growing DNA strand by way of a luciferase enzyme (http://www.454.com). The Roche/454 has the ability to generate longer reads, easier to map onto any reference genomes; however, the technique is prone to Insertion and Deletion errors on the sequences as a result of homopolymeric regions (Margulies et al., 2005; Huse et al., 2007).

In 2007, Solexa GA released the genome analyzer of illumine (http://www.illumina.com). Illumina (Solexa Genome Analyzer (GA)) similarly applies sequencing by synthesis approach and is the most commonly used approach. It is based on a two-step adapter addition and bridge PCR amplification, resulting in clusters that are excited by laser technology (that emits a light signal specific to every added nucleotide), that is detected using CCD camera (coupled-charge device camera) translatable into the nucleotide sequence by computer programs (Shendure and Ji, 2008; Balasubramanian, 2015).

Life Technologies commercialized the Ion Torrent semiconductor sequencer in 2010 (https://www.thermofisher.com/us/en/home/brands/ion-torrent.html). Unlike Roche 454 pyrosequencing that relies on identifying a pyrophosphate, Ion Torrent is similar but relies on detecting hydrogen ions for sequencing (Rotheberg et al., 2011). Moreover, Supported Oligonucleotide Ligation and Detection (SOLiD) by Life Technologies (http://www.lifetechnologies.com) is another Next Generation Sequencing technique. In 2007, Applied Biosystems (ABI) bought SOLiD and developed ABI/SOLID technology that follows Sequencing by ligation (SBL) approach (Shendure and Ji, 2008). The ABI/SOLiD cascade consists of multiple sequencing rounds, starting by adapter addition to the DNA fragments, amplification by PCR emulsion, 8-mer florolabelling and ligation to the DNA fragments. The fluorescence color emitted is recorded and decoded into representative base sequences (Mardis, 2008). Wash and clean is used for the second generation platforms such as SOLiD, Illumina and Roche 454 (Schadt et al., 2010).

Real time sequencers from Pacific biosciences (PacBio) (http://www.pacificbiosciences.com) together with a miniature portable device of Oxford Nanopore MinION (http://www.oxfordnanopore.com) belong to Third generation Sequencing (TGS). This is characterized by two main approaches (Goodwin et al., 2016): Single molecule real time sequencing approach (SMRT) (Bentley et al., 2008) and synthetic approach relying on existing short reads technologies utilized by Illumina (Moleculo) (Harris et al., 2008) in construction of long reads. SMRT approach is the most common, being used in sequencers like Pacific Biosciences and Oxford Nanopore sequencing (MinION-sequencer). Pacific Biosciences uses fluorescent labelling, but instead of PCR amplification, it detects the floro-signals in real time (McCoy et al., 2014; Rhoads and Au, 2015). In Oxford Nanopore sequencing, the initial strand of a DNA molecule is attached to a hairpin on the complementary strand. The fragment goes through a protein nanopore, generating a current disturbance relative to a specific nucleotide base. This is translated into a sequence using a computer software (Mikheyev and Tin, 2014; Laehnemann et al., 2015; Laver et al., 2015). A comparison of the technologies is as shown in Table 2. Despite this availability, studies in Kenya are scanty in application of these techniques for viral pathogenesis in crops.

NGS extraction protocols include: total mRNA (Al Rwahnih et al., 2009; Wylie and Jones, 2011), sRNAs such as siRNAs (Kreuze et al., 2009) and dsRNAs in RNA virus infested material (Coetzee et al., 2010; Dodds et al., 1984). The most commonly utilized approach is total sRNA sequencing (Seguin et al., 2014; Wu et al., 2015). De Novo assembly of siRNAs can be applied to identify both RNA and DNA viruses, though, dsRNAs are only used in the identification of RNA viruses (Seguin et al., 2014; Wu et al., 2015). Virus hunting is faster through metagenomic analysis and deep sequencing, where a lot of known and unknown viruses have been identified from both short and long reads (Capobianchi et al., 2013; Espach et al., 2012).

The direct genetic genome analysis of the environmental sample is called metagenomics (Thomas et al., 2012). This analysis is also termed as ecogenomics as it attempts to sequence total nucleic acids including whole genomes of diseased samples with cheaper purification, cloning and screening steps for identification and diagnosis of viruses (Kreuze et al., 2009; Mokili et al., 2012). The use of metagenomics studies by Studholme et al. (2011) documents that discovery of viruses is possible through Next Generation Sequencing in impacting phylogenesis, pathogenesis and microbial evolution. Next Generation Sequencing approach is cost effective for generating high-throughput data (Marz et al., 2014) and requires no prior knowledge of symptomatic or asymptomatic samples to detect both known and novel viruses. Multiple viruses can be sequenced using Next Generation Sequencing

Table 2. Comparison of Deep sequencing technologies.

Sequencer	Reads per run	Average read length (pb)	Error type	Error rate (%)
Roche 454	100, 200, 400 and 1 M	100, 250, 400, 450 and 700	InDels	1
Illumina	25M-6B	150 and 300	Mismatches	0.1 and 1
SOLiD	3B and 6B	75	Mismatches	-0.1
Ion Torrent	400,000-80 M	200 and 400	InDels	1
PacBio	350, 432, 528, 564 and 660	1300, 2500, 4300, 4600, 8500, 10000 and 13500	InDels	7, 12, 13 and 15
Oxford Nanopore	100	9546	InDels/Mismatches	1.5

Table 3. Applications of next generation sequencing platforms in plant virus discovery.

Viruses, nucleic acid and/or NGS Platform	Plant(s)	Citation(s)
(1) *Cucumovirus.* (i) cDNA	*Gomphrena globose* (Globe amarynth)	Adams et al. (2009)
(2) Cereal yellow dwarf virus, *(i) small RNA (sRNA)* (ii) Roche 454	*Dactylis glomerata* (cocksfoot grass)	Pallet et al. (2010)
(3) Grapevine berry inner necrosis virus (i) Illumina	*Vitis vinifera.* L. (Grapevine)	Giampetruzzia et al. (2011)
(4) Grapevine rupestris stem pitting-associated virus), Hop stunt viroid, Grapevine yellow speckle viroid 1, Grapevine rupestris vein feathering virus and GSyV-1. (i) *small RNA (sRNA)* (ii) Illumina	*Vitis vinifera.* L. (Grapevine)	Giampetruzzia et al. (2011)
(5) Unknown viruses and virus-host interactions (i) NGS	-	Gould and Stinchcombe (2017); Li et al. (2017): Standage et al. (2016); Capobianchi et al. (2013); Prabha et al. (2013) and Studholme et al. (2011).

(Cox-Foster et al., 2007; Quan et al., 2008; Wu et al., 2015). Next Generation Sequencing includes the following steps: sample collection, fractionation, RNA/DNA extraction, DNA/cDNA sequencing, sequence assembly, binning, genome annotation, bioinformatics/statistical analysis, data storage and metadata sharing (Thomas et al., 2012).

Next Generation Sequencing studies have been used to evaluate viruses present in grapevine (Al Rwahnih et al., 2009), to identify unknown viruses (Adams et al., 2009; Coetzee et al., 2010) and in providing deep sequencing viral data of infected plants (Kreuze et al., 2009; Lotos et al., 2017) as shown in Table 3. Similar studies by Coetzee et al. (2010) and Ng et al. (2011) for virus diversity, document the use of Next Generation Sequencing in vector-enabled metagenomics of vector born viruses. This technique has been used for analysis of small interference RNA (siRNA) to identify viruses in infected plants (Kreuze et al., 2009). Also, Adams et al. (2009) used NGS for discovery of novel *Cucumovirus* from long reads of cDNA from a sample of *Gomphrena globosa* infected through mechanical inoculation with an unknown pathogen. Pallett et al. (2010) used Roche 454 pyrosequencing of small RNA (sRNA) from leaves of wild *Dactylis glomerata* (cocksfoot grass), to document novel *Cereal yellow dwarf virus*, in wild cocksfoot grass. Likewise, Giampetruzzia et al. (2011) used Illumina sequencing for discovery of *Grapevine berry inner necrosis virus* (GINV) that was novel and classified as *Grapevine Pinot gris virus*.

NGS of dsRNAs on pooled samples detected numerous grapevine-infecting viruses including putative fungal viruses (Coetzee et al., 2010). Deductions by Giampetruzzia et al. (2011) analyzed small RNA of grapevines in the Trentino region (Italy) using Illumina HTS to discover *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Hop stunt viroid* (HSVd), *Grapevine yellow speckle viroid 1* (GYSVd1), the Marafiviruses *Grapevine rupestris vein feathering virus* (GRVFV) and GSyV-1. Biodiversity studies of viruses by Roossinck et al. (2010) using NGS for Tall Grass Prairie in Northeastern Oklahoma and Northwestern Costa Rica, documented *Potyviridae, Totiviridae, Bromoviridae, Endornaviridae, Luteoviridae Caulimoviridae, Chryso-viridae, Closteroviridae, Narnaviridae, Partitiviridae, Tymoviridae* and some novel viruses. However, no such

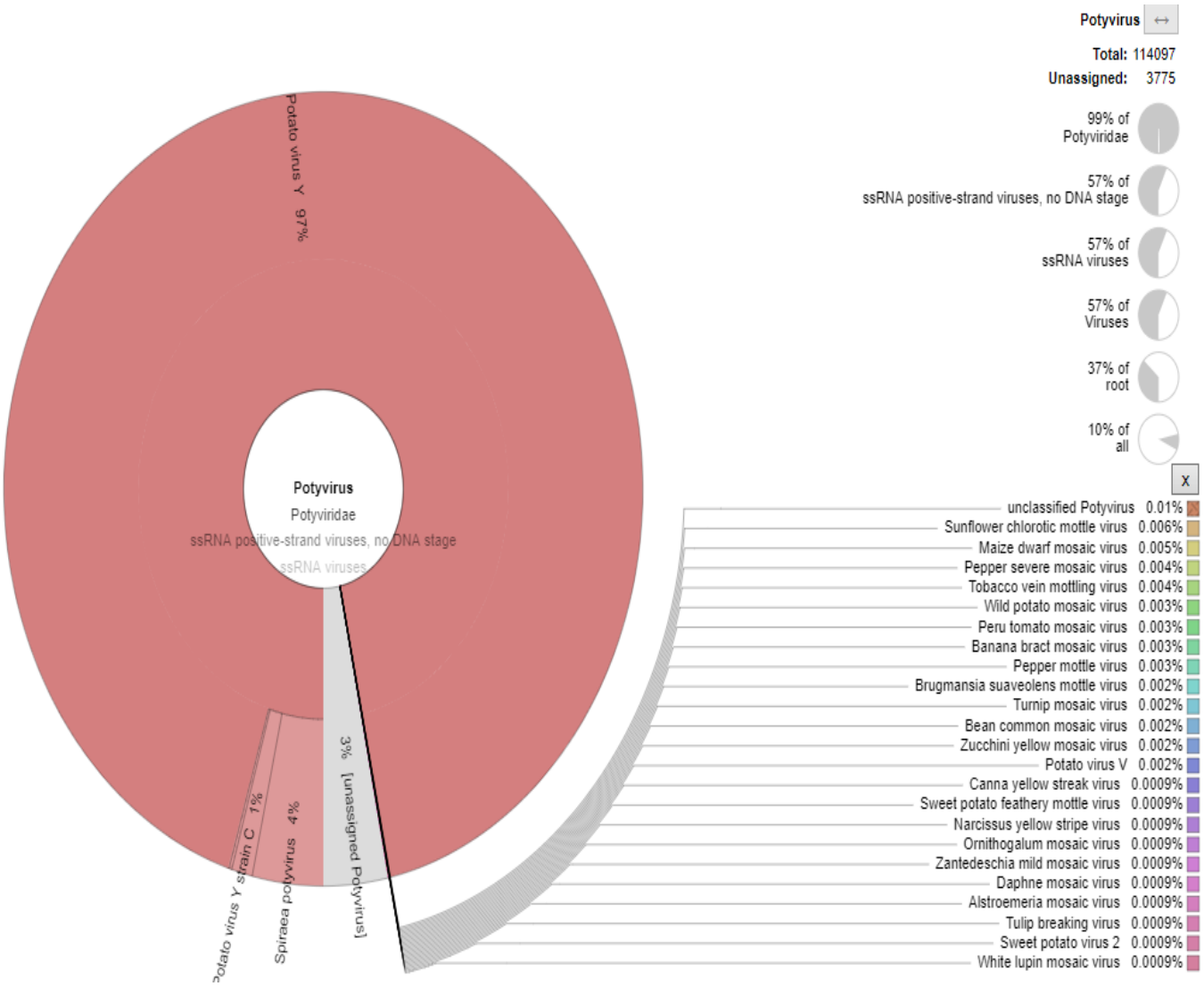

Figure 1. Potyviruses detected using Illumina Sequencer: PVY, PVV, SPFMV, Sunflower chlorotic mottle virus, Sugarcane *mosaic virus,* Pepper mottle virus, Turnip mosaic virus, Watermelon mosaic virus, Watermelon leaf mottle virus, *Papaya ringspot virus, Ceratobum mosaic virus, Chili venal mottle virus, Hippeastrum mosaic virus, Soybean mosaic virus, Banana bract mosaic virus, Lettuce mosaic virus, SPV2* and *Maize dwarf mosaic virus.*

studies have used this technique for discovery and documentation of viruses infecting Irish potato in Kenya.

Genome scanning, genome assembly and De novo genetic mapping can be explored as approaches (Capobianchi, et al., 2013; Gould and Stinchcombe, 2017; Li et al., 2017; Standage et al., 2016). Similarly, Prabha et al. (2013) and Studholme et al. (2011) document applications of HTS to differentiate viral diseases that are unknown and to show virus-host interactions in other plants. Similar studies by Barzon et al. (2011) document the use of Next Generation Sequencing as unbiased, as it needs no antibodies or any prior knowledge of sequence to diagnose. When parallel sequencing is done, the variations that can be determined include: viral genome variations, in-host evolution and virus defense mechanism. An example of viruses detected by Illumina Sequencing is shown on the krona chart Figure 1.

LIMITATIONS OF NEXT GENERATION SEQUENCING

Though sequencing siRNA is sensitive in identifying viruses of varied genome features and different nucleic acid types in low titers not readily detected by other methods, (Kreuze, 2014; Wu et al., 2015), assembly of full genomes or sequence coverage of the viral genome may be difficult (Kreuze, 2014) as small endogenous plant RNAs may interfere with short 21-24 base pairs (Boonham et al., 2014). Next Generation Sequencing

platforms with short read lengths products may equally limit the ability to characterize large repeat regions accurately (Snyder et al., 2010). Lack of known reference genomes for a majority of sequences at times renders classification of reads impossible (Edwards and Rohwer, 2005). However, deep sequenced samples can undergo de novo assembly or mapped to reference genomes for viral discovery (Coetzee et al., 2010; Hwang et al., 2013; Kreuze et al., 2009; Maree et al., 2015). The methods assemble genomes of the majority species in the sample by ignoring technical errors and low-frequency variants. Poor sequence similarity leads to low sequencing depth or coverage to reference sequences as fewer reads cover the same fragment of DNA (Thomas et al., 2012)

CONCLUSION

Potato viruses rely on both mechanical or vector borne transmission modalities. A great number have multiple host ranges across the plant taxa. Studies have shown that, viruses like PSTVd have wild hosts in the *solanaceous* family and other plants. Vectors have the capability of availing the viruses to and from infected or healthy crop irrespectively. Known virus agents can be detected using serology and Polymerase Chain Reaction (PCR). However, the tools are not potent for discovery or novelty studies aiming to detect undocumented virome. Next generation sequencing is an emerging tool to plant molecular biologists in determining whole virus genomes, and undertaking viral metagenomic studies for novel viruses. Serology and PCR diagnostics have had challenges in detecting unknown virus agents. These techniques are extremely costly per sample other than being time consuming to run. With the constant change in genetics of viruses, novelty and un-targeted viruses are missed out. Next Generation Sequencing, through sample pooling by RNA*taq-Seq* protocols, is able to analyse numerous samples in a single barcoded run. This cuts down costs as samples are run simultaneously, saves time as the various platforms have a higher throughput rate and increases robustness by being able to detect both known and unknown virome, leading to discovery. Adopting Next Generation Sequencing to boost serology and PCR diagnostics will allow for total documentation of viral entities infecting Irish potato in Kenya. Virus disease etiology will be opened and an understanding of virus-host interactions enabled. Furthermore, antagonistic and synergistic or mutualistic virus relationships will be opened up upon determination of the total virome.

RECOMMENDATIONS

Serology relies on familiarity for it to be effective, hence would be robust with up to date viral genomes. This is also important for Polymerase Chain Reaction (PCR) and PCR variants used in the Molecular diagnostics of Irish potato viruses. Next Generation Sequencing has justified ability in cataloguing familiar and new novelties in the world of virome discovery. This can be adopted and used to avail information on the potato viruses in the country. Disease dynamics have to be conducted to ascertain causality of disease Vis a Vis the viral agent tied to it. Since Next Generation Sequencing avails copious data and metadata on genome discovery within a sample, it is prudent for further studies to be conducted on disease symptomatology, virus-host interactions and virus-vector mediation to determine the pathology. Furthermore, stacked viral influence and effect on symptomatology and infection modalities in potatoes has to be conducted as an extrapolation of disease dynamics. This is to rule out misdiagnosis and enhance documentation of viral interactions in potato hosts. It is mandatory to conduct a countrywide potato viral discovery studies using deep massively parallel Next Generation Sequencing techniques such as: Illumina, SOLiD, Roche 454 pyrosequencing, Ion Torrent, Oxford Nanopore and Pacific Biosciences sequencing technologies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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