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Ravinder Kumar
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Rahul Kumar Tiwari
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Jeevalatha A
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Priyanka Kaundal
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Sanjeev Sharma
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

SK Chakrabarti
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Corresponding Author:
Ravinder Kumar
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Potato viruses and their diagnostic techniques: An overview

Ravinder Kumar, Rahul Kumar Tiwari, Jeevalatha A, Priyanka Kaundal, Sanjeev Sharma and SK Chakrabarti

Abstract
Potato (*Solanum tuberosum* L.) is a vulnerable host of several plants viruses and viroid resulted severe yield losses. The viruses and viroid cannot be controlled therapeutically as there is no direct chemical available globally. Hence, in the first instant their control relies on inhibit the establishment, evolution and dispersal of the causal viruses, and to prevent them, the effective detection technique is much important. The development of reliable and specific detection methods is a compulsion and strong virus indexing is the only way to produce healthy seed potato. Detection of potato viruses is becoming more crucial because of globalization of trade by free trade agreement and therefore, a concern for national quarantine services to ensure the safe movement of potato germplasm across the border. The identification and detection of viruses is not an easy task as in case of fungi and bacteria, they can be seen only in a transmission electron microscope. Potato viruses are host specific and for accurate identification, appropriate detection method from sampling to final step must be deployed. In this review, several methods for virus and viroid detection are discussed with more emphasis on recently developed molecular diagnostic techniques. Molecular diagnostic techniques may be the choice for disease free seed potato production but for epidemiological and aetiological studies, biological indexing, serological and electron microscopy techniques are essential. This exercise will be helpful in sustainable agriculture and reliable health monitoring of potato as well as added knowledge to the researchers for adequate utilization of these techniques.

Keywords: Serology, indicator host, nucleic acid, potato virus, PCR, microarray, detection

Introduction
Potato (*Solanum tuberosum* L.) is an important cultivated crop takes third place in the world after rice and wheat in terms of human consumption. Global annual potato production during 2017 was 388 million tonnes from 19.30 million hectare area with average productivity of 20.11 tonnes/hectare. India is second major potato producer in terms of fresh production after China. India produces 48.60 million tonnes from 2.17 million hectare area with average productivity of 22.30 tonnes/hectare (FAOSTAT 2017). The rapidly growing human population is a challenge to the whole world to provide food security in near future. To combat the situation, increase in total production and productivity is only the way left for us, but high productivity and production has a synergistic effect on increase in diseases intensity. Potato is most favourable host of more than three dozen plant viruses. The viruses are tiny plant pathogens, made of a coat protein and nucleic acid (DNA or RNA) and viroid as the smallest plant pathogen made of the only RNA. Symptoms/disease caused by potato viruses and there genome structure, mode of transmission and distribution pattern have been summarized in brief in Table 1 [1, 2, 3, 4]. At global scale, exact data concerning economic losses caused by potato viruses is lacking but losses due to plant viruses have been estimated more than millions of dollars per year globally [5]. A rough data shows that potato viruses may cause up to 50% loss in tuber yield [2, 6, 7, 8]. Generally tuber yield losses are reported 5-15%, if all plants are secondarily infected with PVX and PVS; 15-30% for 100% secondary infection of *Potato virus Y* strain N (PVY^n) and 40-70% due to infection of PLRV [9, 10, 11]. Besides, in Europe and North America PSTVd is well known to reduce yields greatly (16-64%) depending on the viroid strain/potato variety and warm weather [12]. The severe strains of PVY and PLRV have the potential to reduce yield up to 80%, while mild viruses like PVX, PVS and *Potato virus M* (PVM) can cause up to 30% yield loss [8]. A tospovirus *Groundnut bud necrosis virus* (GBNV) causing severe stem/leaf necrosis disease in plains/plateaux of central/western India heavily infects the potato [13, 14, 15, 16]. Similarly, in India a whitefly transmitted begomovirus ToLCNDV-potato known to cause apical leaf curl disease in potato, has become a serious problem and up to 40–75% of infections were found in the traditional cultivars grown in India and yielded high losses [17, 18, 19, 20].

Table 1: Important characteristics, genome structure, mode of transmission and distribution of potato viruses and viroid.

Disease/symptoms	Virus (acronym)	Virus genus/group	Family	Morphology/number of distinct particle size	Particle diameter	Vectors	Mode of transmission, spread	Geographical distribution
Potato leaf roll	*Potato leafroll virus* (PLRV)	*Polerovirus* Group IV (+)ssRNA	*Luteoviridae*	Isometric/01	24	Aphid[P]	TPS	Worldwide
Faint/latent mosaic	*Potato virus X* (PVX)	*Potexvirus* Group IV (+)ssRNA	*Flexiviridae*	Filamentous/01	13	-	Contact, TPS	Worldwide
Necrotic symptoms on potato	*Potato virus Y* (PVY)	*Potyvirus* Group IV (+)ssRNA	*Potyviridae*	Filamentous/01	11	Aphid[NP]	TPS, mechanical	Worldwide
Mild mosaic	*Potato virus A* (PVA)	*Potyvirus* Group IV (+)ssRNA	*Potyviridae*	Filamentous/01	-	Aphid[NP]	Mechanical	Worldwide
Mottle, mosaic, crinkling and rolling symptoms	*Potato virus M* (PVM)	*Carlavirus* Group IV (+)ssRNA	*Flexiviridae*	Filamentous/01	12	Aphid[NP]	Contact	Worldwide
Mottling and bronzing	*Potato virus S* (PVS)	*Carlavirus* Group IV (+)ssRNA	*Flexiviridae*	Filamentous/01	12	Aphid[NP]	Contact	Worldwide
Potato apical leaf curl disease	Tomato leaf curl New Delhi *virus-potato* (ToLCNDV-potato)	*Begomovirus* Group II (ssDNA)	*Geminiviridae*	Geminate particles	21-24 nm	Whitefly	-	India
Dark streak and wilting on stem	*Tomato spotted wilt virus* (TSWV) or Peanut bud necrosis virus	*Tospovirus* Group IV (-)ssRNA	*Bunyaviridae*	Enveloped particle/01	70-110	Thrips[P]	Mechanical	Hot climate, Worldwide
Necrotic, leaf deformation and mosaic	*Potato aucuba mosaic virus* (PAMV)	*Potexvirus* Group IV(+)ssRNA	*Flexiviridae*	Filamentous/01	11	Aphid[HC]	TPS, Contact	Worldwide (Uncommon)
Yellow blotching on leaves	*Alfalfa mosaic virus* (AMV) *	*Alfamovirus* Group IV (+)ssRNA	*Bromoviridae*	Bacilliform/ 04-05	19	Aphid[NP]	TPS, Pollen	Worldwide (Uncommon)
Mild mosaic, chlorotic netting, rugosity	*Andean potato latent virus* (APLV) *	*Tymovirus* Group IV (+)ssRNA	*Tymoviridae*	Isometric/01	28-30	Flea Beetle	TPS, Pollen	S-America
Mild or severe mottle	*Andean potato mottle virus* (APMV) *	*Comovirus* Group IV (+)ssRNA	*Comoviridae*	Isometric/01	28	Beetle	Contact	S-America
Arracacha or oca	*Arracacha virus B* - Oca strain (AVB-O) *	*Nepovirus* Group IV (+)ssRNA	*Sequiviridae*	Isometric/01	26	Unknown	TPS, Pollen	Peru, Bolvia
Mosaic disease	*Cucumber mosaic virus* (CMV) *	*Cucumovirus* Group IV (+)ssRNA	*Bromoviridae*	Isometric/01	30	Aphid[NP]	Sap, TPS	Worldwide (Uncommon)
Black ring spot	*Potato black ringspot virus* (PBRSV) *	*Nepovirus* Group IV (+)ssRNA	*Comoviridae*	Isometric/01	26	Nematode[SP]	Soil borne, TPS, Pollen	Peru
Leaf distortion with yellow blotch	*Potato deforming mosaic virus* (PDMV) *	*Begomovirus* Group II (ssDNA)	Geminiviridae	Segmented/02	18	Whitefly[SP]	TPS	Brazil
Necrotic and vein clearing	*Potato latent virus* (PotLV) *	*Carlavirus* Group IV (+)ssRNA	*Betaflexiviridae*	Filamentous/01	-	Aphid[NP]	Contact	N-America
Corky ringspot or spraing disease	*Tobacco rattle virus* (TRV) *	*Tobravirus* Group IV (+)ssRNA	*Virgaviridae*	Rod or tubular/02	22	Nematode[P]	Mechanically, TPS	Worldwide
Mottling and necrosis	*Tobacco streak virus* (TSV) *	*Ilarvirus* Group IV (+)ssRNA	*Bromoviridae*	Quasi-isometric/01	22-35	Thrips	Pollen, TPS, mechanical	S-America
Yellow dwarf of potato	*Potato yellow dwarf virus* (PYDV) *	*Nucleorhabdovirus* Group V ((-)ssRNA)	*Rhabdoviridae*	Bacilliform	75	Leafhopper[P]	Mechanical	N-America
Yellow mosaic and stunting	*Potato yellow mosaic virus* (PYMV) *	*Begomovirus* Group II (ssDNA)	*Geminiviridae*	Segmented/02	18-20	Whitefly[SP]	-	Caribbean region
Blotching or mottling, Spraing disease	*Potato mop top virus* (PMTV) *	*Pomovirus* Group IV (+)ssRNA	*Virgaviridae*	Rod or tubular/02	18-20	Fungus[P]	Mechanical	W-Europe and S-America

Yellowing disease of potato	*Potato yellow vein virus* (PYVV) *	*Crinvirus* Group IV (+)ssRNA	*Closteroviridae*	Filamentous	-	Whitefly P	Infected tuber	S-America
Yellowing symptoms	*Potato yellowing virus* (PYV) *	*Alfamovirus* Group IV ((+)ssRNA	Bromoviridae	Bacilliform	21	Aphid SP	TPS	S-America
Mild leaf mottle and latent	*Potato virus T* (PVT) *	*Trichovirus* Group IV (+)ss RNA	*Flexiviridae*	Filamentous/01	12	-	Contact, TPS, Pollen	S-America
Necrotic spotting	*Potato virus U* (PVU) *	*Nepovirus* Group IV (+)ssRNA	*Comoviridae*	Isometric/01	28	Nematode	Contact, TPS	Peru
Necrotic spot	*Potato virus V* (PVV) *	*Potyvirus* Group IV (+) ss RNA	*Potyviridae*	Filamentous/01	12-13	Aphid NP	TPS	N-Europe, S-America
Apical leaf curl	*Solanum apical leaf curling virus* (SALCV)*	*Begomovirus* Group II (ssDNA)	*Geminiviridae*	Segmented/03	18	Whitefly SP	TPS	Peru
Mosaic disease	*Tobacco mosaic virus* (TMV) *	*Tobamovirus* Group IV (+)ssRNA	*Virgaviridae*	Rod or tubular/01	18	Fungus	Contact, Infected soil	Worldwide
ABC disease of potato	*Tobacco necrosis virus* (TNV) *	*Necrovirus* Group IV (+)ssRNA	*Tombusviridae*	Isometric/01	26	Fungus P	Soil borne spores, mechanical	Europe N-America
Chlorotic mottling and/or ringspots in leaves	*Tomato black ring virus* (TmBRV) *	*Nepovirus* Group IV (+)ssRNA	*Comoviridae*	Isometric/02	5-6	Nematode P	Pollen, TPS	Europe
Yellow molting and mosaic	*Tomato mosaic virus* (ToMV) *	Tobamovirus Group IV (+)ssRNA	*Virgaviridae*	Rod or tubular/01	18	-	TPS, Pollen, Contact	Hungary
Colour change in foliage, small leaves and spindal like elongation	*Potato spindle tuber viroid* (PSTVd)	*Pospiviroid* Circular (+)ssRNA	*Pospiviroidae*	Circular ss RNA only		Aphid CI	TPS, Pollen, Contact	United states, Canada, South Africa, Russia

TPS = True potato seed; P/NP = Persistently/Non-persistently transmitted; SP =Semi-persistently transmitted; HC =Helper component involved for transmission, CI = Co-infection of PLRV essential for aphid transmission of viroid; * = Viruses that are of quarantine importance in India or not reported in potato in India

Transboundary movement of potato germplasm has significantly increased in this era due to globalization of trade. But it puts a lot of pressure on national quarantine services to accurately detect virus infection in tubers and seed in order to ensure safer transboundary movement. Methods for plant virus diagnosis have evolved in parallel to the progress in the knowledge of these components. There are two broad categories of virus diagnostics: one is related to biological property in terms of relation of virus with its host and vector and other is intrinsic property of virus itself [2, 21 22]. Detection methods relying on coat protein included precipitation/agglutination tests, enzyme-linked immunosorbent (ELISA) assays, and immunoblotting [2, 23], while nucleic acid-based techniques like polymerase chain reaction, LAMP and dot-blot assays are more sensitive than other methods [24, 25, 26, 27]. During the last two decades, there has been tremendous progress on nucleic acid based diagnostics and has subsequently revolutionized the potato virus diagnostics. Furthermore, we are at the age of genomics, in which entire DNA or RNA sequences of organisms and their genetic mapping are being determined which provides the sufficient data for micro-array based detection of potato viruses [28]. Thus, we are immersed in this fascinating era, with a fast developing present and a hopeful future of new possibilities. Rapid development of modern diagnostic tools provides greater flexibility, high sensitivity and specificity for timely diagnosis of viral diseases. This will help in epidemiological studies, post entry quarantine, disease monitoring, seed potato certification, and advanced virus resistant breeding programs. Nevertheless, deployment of these techniques to address the problems due to viral diseases in potato depends on having appropriate research facilities and critical scientific proficiency. An overview of various methods available for the detection of potato viruses is provided in the following sections with emphasis on how they can be utilized by scientists in developing countries like India. This review mainly focuses on the modern molecular detection techniques developed in relation to potato viruses and viroid and their applicability. The effective management strategies for potato virus recognize the availability of a robust, cheap and reliable technique for sustainable agriculture.

Conventional methods of diagnosing potato viruses

Traditional methods of virus disease diagnosis and detection correspond to symptomatology, biological indexing, transmission, electron microscopy and serology were known and deployed as backbone in healthy seed potato production, quarantine and certification programme [2, 20, 29, 30]. Initially, the viruses can be readily detected through their reaction on the indicator hosts [31]. Biological indexing was successfully used for PVA identification and detection [32]. Although symptoms developed on susceptible indicator host plants are considered sufficient up to some extent but it had a lacuna as in case of exact identification of viruses. For identification of unknown viruses and its strains, host range studies have a considerable impact because of characteristics symptoms development [2, 33, 34]. A large number of indicator host plants are known like *Nicotiana tabacum, N. clevelandii, N. glutinosa, N. debneyi, Solanum tuberosum, Physalis*

floridana, Phaseolus vulgaris, Lycopersicon esculantum, Datura stramonium, D. metel, Gomphrena globosa, Chenopodium amaranticolor, C. hybridum, C. ambrosioides, C. murale, C. quinoa, C. opulifolium, C. polyspermum, C. rubrum and *C. urbicum, Cyamopsis tetragonoloba, Trifolium incarnatum, Cucumis sativus* for virus identification as systemic infection or local lesions. These plants grow *in vitro* for experimental purposes but they are not suitable to test a large number of samples and were considered more time consuming. The main hurdle in this diagnostic method is production of different kind of symptoms in different indicator host of the same virus or its strain. With the success of hybridoma technology to produce antibodies first time against TMV, serological techniques were extended and popularized for diagnostic of potato viruses [35] and revolutionized the virus indexing process with the help of different serological techniques like chloroplast agglutination, micro-precipitation tests and gel immune diffusion [36]. Later these techniques were exploited for diagnosis of 50 different plant viruses, including important potato viruses such as PVX, PVY, PVA, PVM, PVS and PLRV [2, 37, 38]. Consequently, to increase the sensitivity of serological methods, a solid phase ELISA was developed and has secured significant place in potato virus detection for a long time. The reason for its widespread adaptability is easy of doing; high sensitivity and multiple sample analysis in one go. The minimum level of virus titer needed for detection by ELISA is approximately 2ng/ml. ELISA has been developed for detection of PLRV in single aphid [39]. DAS-ELISA was successfully detected PVY [40]. DoT-ELISA has been used to detect PLRV [41], PYX, PVS and PVY [42]. Both PVY and PVX have been detected from tubers using tissue blot Immunoassay [43], latex agglutination [44] for mass testing followed by Immunosorbent electron microscopy (ISEM) for specific detection of low concentration of PLRV in potato nucleus stocks/mericlones [10, 35, 45, 46, 47]. Although, serological tests are enough virus-specific [38, 48, 49], but the production of antibodies is often labor-intensive. However, electron microscopy is considered highly sensitive and specific to detect potato viruses with the advantage of morphological determination [37, 50].

Nucleic acid based methods of diagnosing potato viruses

As it was felt that the virus testing is more crucial step for healthy seed production and virus management in potato, a highly specific, sensitive, robust, simple and cost-effective technique must be developed. To overcome the fact nucleic acid based techniques were exploited and in this series, PCR offers several advantages because of high accuracy, sensitivity and specificity to detect potato viruses [27, 34]. In recent years, PCR and RT-PCR are more popular techniques for detection and identification of potato viruses. In case of RNA viruses, a cDNA strand which is complementary to the virus is made with reverse transcriptase (RT). RT-PCR is the "gold standard" molecular method used for the detection of potato viruses due to its high sensitivity and specificity. In terms of sensitivity researcher claims that RT-PCR is 1000 times sensitive than ELISA [51]. Immunocapture PCR (IC-PCR) captures PLRV particles by antibodies with amplification by PCR [51]. Direct binding RT-PCR (DB-RT-PCR) was used for detection of PVY [52]. In Print-capture PCR (PC-PCR) there is no need for sample grinding as it does not affect sensitivity. This method was used for detection of PVY and ToLCNDV [51, 53], PLRV [54]. Nested PCR, a variant of PCR was used as a sensitive and highly specific in detection of many potato viruses [55]. Molecular detection techniques are more reliable, specific, sensitive and inexpensive compared to conventional techniques [12, 27, 56] and potato viruses such as PVM, PVS, PVA, PVX, PVY and PLRV have been detected using molecular technique like RT-PCR, multiplex PCR, real-time PCR, reverse transcription-loop-mediated isothermal amplification (RT-LAMP) and microarray [26]. Likewise, in earlier days, the biological indexing or bioassay was used for a long time for viroid detection in potato because serological methods did not worked as viroid genome have only RNA [29, 30, 57]. Similarly, with the development of virus diagnostic, a more precise, reliable and rapid techniques polyacrylamide gel electrophoresis (PAGE) was developed for viroid detection [58]. PAGE technique was very specific due to its characteristic as according to the size, separation of nucleic acids could be possible based on differential mobility in an electric field and proved for successful detection of viroid [29, 30, 59]. Therefore, the PAGE was successfully used for diagnosis of PSTVd in potato [60, 61, 62]. First ever molecular hybridization was done in potato to detect PSTVd [63]. However, the nucleic acid spot hybridization (NASH) replaced the PAGE because of 1000 times sensitiveness [2, 12, 61]. The whole process of NASH also referred as dot blot hybridization involves solid-liquid hybridization. The detection of three major potato viruses PVY, PVX and PLRV has been reported by using radioactive labeled complementary DNA (cDNA) probes [64]. Nonradioactive, biotinylated RNA and DNA probes for PVX and PVS in crude potato extract have been reported [65, 66]. PLRV detected by dot-blot hybridization [67]. From the crude extract of potato plant PVX, PLRV, PSTVD and PVY were detected using Dot-blot assay [68, 69]. Later on viroid detection was also improved by use of nucleic acid-based techniques. The different nucleic acid based molecular methods for detection of potato viruses and viroid has been summarized in table -2.

Table 2: Method for detection of viruses and viroid in potato.

S. No	Virus	Technique	Reference
	PVA	RT-PCR	Cerovska *et al*. 1998; Singh and Singh 1998; Collins *et al*. 1993 [70, 71, 72]
	PVS	RT-PCR	Kaushal *et al*. 2007; Zhou *et al*. 2007; Matoušek *et al* 2000; Salama and Saghir 2017 [73, 74, 75, 76]
	PVM	RT-PCR	Huimin *et al*. 2010 [77]
	GBNV	Print capture RT-PCR	Kaushal *et al*. 2010 [78]
		RT-PCR	Pundhir *et al*. 2012; Raigond *et al*. 2017; Akram 2003 [15, 16, 79]
	PLRV	RT-PCR	Hadidi *et al*. 1993; Singh *et al*. 1995; Singh *et al*. 1997; Mukherjee *et al*. 2003; Jeon *et al*. 1996; Spiegel *et al*. 1993 [80, 81, 82, 83, 84, 85]
		IC-RT-PCR	Leone *et al*. 1997; Ahouee *et al* 2010; Schoen *et al*. 1996; Hemmati *et al*. 2010 [51, 54, 86, 87]
		One step RT-LAMP	Ahmadi *et al*. 2013 [88]
		RT-LAMP	Almasi *et al*. 2013a,b; Ju 2011 [89, 90, 91]
		Squash print RT-LAMP	Raigond *et al*. 2019 [92]

		Multiplex AmpliDet RNA	Klerks *et al*. 2001 [93]
	PVX	RT-PCR	Jamal *et al*. 2012, Jeevalatha *et al* 2016; Mandal *et al*. 2012; Massumi *et al*. 2014; Nosheen *et al*. 2013; Soliman *et al*. 2000; Yu *et al*. 2008; Abbas and Hameed 2012 [94, 95, 96, 97, 98, 99, 100, 101]
		One step RT-LAMP	Raigond *et al*. 2019[102]
	PVY	RT-LAMP	Jeong *et al*. 2015 [27]
		RT-PCR	Singh and Singh 1996; Singh and Singh 1997; Barker *et al* 1993; Hu *et al*. 2009; Ghosh and Bapat 2006; Xu *et al*. 2005 [103, 104, 105, 106, 107, 108]
		Three primer PCR	Moravec *et al*. 2003[109]
		IC-RT-PCR	Gawande *et al*. 2011; Juil *et al*. 2016 [52, 110]
		RT-PCR & Real Time PCR	Mackenzie *et al*. 2015; Fox *et al*. 2005 [40, 111]
		One step RT-LAMP	Przewodowska *et al*. 2015 [112]
		SNP based technique	Jacquot *et al*. 2005 [113]
		RT-LAMP	Nie 2005 [114]
		Immunocapture RT-LAMP	Almasi and Dehabadi 2013 [89]
	ToLCNDV	Print Capture PCR	Gawande *et al*. 2007 [53]
		Uniplex and duplex PCR	Jeevalatha *et al*. 2013 [20]
		RCA-PCR	Jeevalatha *et al*. 2013 [115]
		PCR	Sridhar *et al*. 2016 [116]
		LAMP	Jeevalatha *et al*. 2018 [117]
	PSTVd	PAGE	Diener and Smith 1971 [118]
		Dot-blot hybridization	Owens and Diener 1981; Podleckis *et al*. 1993, Mumford *et al*. 2000 [63, 119, 120]
		Tissue blot hybridization	Podleckis *et al*. 1993 [119]
		R-PAGE	Roenhorst *et al*. 2000; Owens *et al*. 2012 [121, 122]
		Dot and print RT-PCR	Weidemann and Buchta 1998 [123]
		RT-PCR	Shamloul and Hadidi 1999; Mumford *et al*. 2000 [125, 124]
		Duplex/Multiplex RT-PCR	Nie and Singh 2001; Hataya 2009 [125, 126]
		Multiplex RT-PCR	Shamloul *et al*. 2002 [127]
		RT-LAMP	Tsutsumi *et al*. 2010, Lenarcic *et al*. 2013 [128, 129]
		RT-qPCR	Mumford *et al*. 2000 [124]
		Real Time RT-PCR	Boonham *et al*. 2004 [130]
	PYVV	RT-PCR	López *et al*. 2006 [131]
	PMTV	RT-PCR	Xu *et al*. 2004 [108]
	PLRV, PVY	RT-PCR	Hogue *et al*. 2006; Russo *et al*. 1999 [132, 133]
	PMTV, TRV	Multiplex Real-Time Fluorescent RT-PCR	Mumford *et al*. 2000 [124]
	PVY and PLRV	One-step triplex RT-PCR	He *et al*. 2006 [134]
	PMTV, TRV, PVY^{NTN}	multiplex real- time PCR (TaqMan)	Boonham *et al*. 2000 [135]
	PYVV, TRV and TICV	Multiplex RT- PCR	Wei *et al*. 2009 [136]
	PLRV, PVY, PVX	RT-PCR	Saikhan *et al*. 2014 [137]
	PVX, PLRV and PVS	RT-PCR	Lacomme *et al*. 2015 [138]
	PVY and PVS	Duplex RT- PCR	Raigond *et al*. 2013 [139]
	PVA and PVM	Duplex RT- PCR	Meena *et al*. 2017 [140]
	PVY, PVX, PLRV	Multiplex microsphere immunoassay (MIA)	Bergevoet *et al*. 2008 [141]
	PVS, PLRV, PVX and PVY	Multiplex RT-PCR	Bostan *et al*. 2009 [142]
	PVS, PVX, PVY, and PLRV	Multiplex RT-PCR	Singh *et al*. 2004 [143]
	PAMV, PLRV, PVM, PVS, PVX	Multiplex RT-PCR	Kumar *et al*. 2017 [144]
	PVY, PVX, PLRV	Multiplex RT-PCR	Bergervoet *et al*. 2008 [141]
	PVA, PVX, PVY, PLRV, PVS	Multiplex RT-PCR	Du *et al*. 2006 [145]
	PLRV, PVX, PVY	MultiplexRT- PCR	Verma *et al*. 2003 [146]
	PVY, PVX, PLRV	Multiplex RT- PCR	Shalaby *et al*. 2002 [147]
	PVS, PVX, PVY, PLRV, PSTVd	Multiplex RT- PCR	Peiman and Xie 2006 [148]
	PVA, PLRV, PVY, PVX, PVS	Uniplex and multiplex RT-PCR	Nie and Singh 2000 [149]
	PLRV, PVA, PVX and PVY	Real Time PCR	Agindotan *et al*. 2007 [150]
	PVY^{O}, PVY^{N}, PVY^{C} and PVY^{NTN}	RT-PCR	Boonham *et al*. 2002 [151]
	PVY^{O}, PVY^{N}, PVY^{NTN}, $PVY^{N:O}$, $PVY^{N}/^{NTN}$	Multiplex RT-PCR	Lorenzen *et al*. 2006 [21]
	$PVY^{N,}$ PVY^{O} $PVY^{C,}$ $PVY^{N/NTN}PVY^{N\text{-}w}$, $PVY^{NTN,}$	Duplex and multiplex RT-PCR	Schuber *et al*. 2007 [152]
	PVY^{NTN}	RT-PCR	Moravec *et al*. 2003 [109]
	PVY and serotypes O and N	Multiplex RT-PCR	Chikh *et al*. 2008 [153]
	$PVY^{N:O}$, PVY^{NTN}	Multiplex RT- PCR	Nie and Singh 2003 [154]
	$PVY^{O}PVY^{N}$, PVY^{C}, PVY^{NTN}, $PVY^{N}Wi$	Multiplex RT-PCR	Rigotti and Gugerli 2007 [155]

	PVY^{O}, EU-PVY^{N}/^{NTN}, NA-PVY^{N} and NA-PVY^{NTN}	Uniplex and Multiplex RT-PCR	Nie and Singh 2002 [156]
	PVY^{NTN-NW}, SYR-III, PVY^{O}, PVY^{N}, PVY^{NTN} and $PVY^{N}W$	Multiplex RT-PCR	Ali *et al*. 2010 [157]
	PVY^{O}, $PVY^{N:O}$, PVY^{N}, EU-PVY^{NTN}, NA-PVY^{NTN}	RT & IC-RT-PCR Multiplex PCR	Malik *et al*. 2012 [158]
	$PVY^{N}Wi$	RT-PCR	Kamangar *et al*. 2014 [159]

Advanced nucleic acid based method for diagnosis of potato viruses

An advancement in nucleic acid based detection for virus and viroid was carried out simultaneously in potato. Many viruses at a time infect a single crop or host and potato crop was not an exception. Multiplex PCR/RT-PCR was proved as important technique to detect several viruses in a single go. There are several findings multiple potato viruses detection in single reaction like PLRV detection by duplex RT-PCR [12], multiplex detection of PVS, PLRV, PVX, PVA, PVY, and PSTVd [149], PVY, PVX, PLRV and PSTVd in potato leaves [160], PYVV, *Tomato infectious chlorosis virus* and TRV in potato leaves were detected by a multiplex RT-PCR assay [136]. Real-time PCR was developed because of its advantage as it requires fewer reagents and less time, and also allows additional studies to be performed during detection, quantification of original target population, detection of several variants of a virus or point mutations in a general. A multiplex real-time RT-PCR was developed for detection of TRV and PMTV in potato tubers [120], PYVV [131]. PVY detected in potato tubers by using real time RT-PCR [40]. PVY and PLRV were detected by multiplex real-time RT-PCR using molecular beacons [93]. A real-time RT-PCR was developed for detection of four important potato viruses [150]. More recently 725 tuber and 1025 leaf samples were analyzed and confirmed the occurrence of potato viruses Y, PVS, and PVM [161].

Mostly plant viruses are having RNA although geminiviruses infecting vegetables including potato having DNA in their genome. Geminiviruses viruses DNA is circular in nature and can be easily detected using Rolling Circular Amplification (RCA) technique. This technique has efficiently characterized several geminivirus genome components using restriction fragment length polymorphism (RFLP) analysis [162]. A highly robust RCA-PCR method was developed for detection of ToLCNDV in potato [115]. Subsequently, Southern blotting method has been used for quantitative determination of many begomoviruses like ToLCNDV an emerging virus in potato in India [163, 164]. More recently developed methods are reverse transcriptase loop-mediated isothermal amplification (RT-LAMP), micro-and macroarrays and next-generation sequencing (NGS), revolutionized virus and viroid detection due to faster and sensitiveness [59, 122, 165]. LAMP is cost effective and user-friendly and can be carried out in a simple laboratory setup using a water bath or heat block. RT-LAMP assays are available for the detection of PLRV [88], PVY and PVX [89, 112]. First time reported for the print-capture LAMP assays for specific detection of ToLCNDV [117]. Microarrays are modern laboratory tool comprising of thousands of specific probes spotted onto a solid surface (usually nylon or glass). The probes are made in such a way that those are complementary to a specific DNA sequence (genes, ITS, ribosomal DNA). This detects at a time expression of thousands of genes and has been used for several potato viruses' detection (Table 3).

Table 3: Potato viruses' detection by macro or microarray techniques.

Abbreviation	Plant host/propagation host	References
PLRV	*Solanum tuberosum*	Agindotan and Perry 2007; Agindotan and Perry 2008; Maoka *et al*. 2010; Nicolaisen, 2011; Wang *et al*. 2012 [161, 166, 167,168, 169]
PMTV	*N. benthimiana*	
PVX	*N. tabacum, N. benthamiana Solanum tuberosum*	
TSWV	Lobelia *N. benthamiana*	Maoka *et al*. 2010; Nicolaisen *et al*. 2011[167,168]
PAMV	*N. occidentalis*	Agindotan and Perry 2007; Agindotan and Perry 2008; Maoka *et al*. 2010; Wang *et al*. 2012 [161, 166, 167, 169]
PVA	*N. benthamiana, S. tuberosum*	
PVM	*N. occidentalis, S. tuberosum*	
PVS	*N. occidentalis S. tuberosum*	
PVY	*N. benthamiana, S. tuberosum*	
ToRSV	*N. benthamiana*	
PotLV	*S. tuberosum*	Agindotan and Perry 2008[166]
TRV	*S. tuberosum*	

Pyrosequencing is a method of DNA sequencing based on the "sequencing by synthesis" principle. Unlike Sanger sequencing it depends on pyrophosphate release on nucleotide incorporation and being exploited in potato virus diagnostics [166]. A wide range of diagnostics methods have been developed for identification and detection of potato viruses and viroid. The strength and applicability of these methods relies on simplicity, precision, robustness, reproducibility and cost effectiveness. Comparison of sensitivity, specificity, feasibility, rapidness and cost of different viruses and viroid detection techniques has been analyses and summarized in table 4.

Table 4: Comparison of sensitivity, specificity, feasibility, rapidness and cost of different nucleic acid based techniques in detection of potato viruses.

Technique	Sensitivity[a]	Specificity[b]	Feasibility[c]	Rapidness[d]	Cost[e]
Molecular hybridisation	Low	Moderate	Complex	Time taking	High
Conventional PCR	Medium	Moderate	Easy	Quick	Medium
Nested PCR in a single tube	High	Moderate	Easy	Time taking	Medium
Cooperational-PCR	High	Moderate	Easy	Quick	Medium

Multiplex PCR	Medium	Moderate	Easy	Quick	Low
Multiplex nested PCR	High	Moderate	Complex	Time taking	Medium
Real-time PCR	Very high	High	Easy	Very Quick	High
Microarrays	Medium	High	Complex	Time taking	Very high
RT-LAMP/ LAMP	Very high	High	Very easy	Very Quick	Low

[a] Sensitivity-Low, medium, high and very high
[b] Specificity-Less, moderate and high
[c] Feasibility -Easy, very easy and complex
[d] Rapidness – Time taking, quick and very quick
[e] Cost – Low, medium, high and very high

Conclusions

A plethora of detection and identification techniques are currently available for the potato viruses and being used in healthy potato production system as per need and feasibility. These techniques are routinely useful in survey and monitoring of viral diseases, seed certification, post entry quarantine systems, epidemiological studies and advanced breeding targeting host plant resistance [2, 117, 144]. The exploitation of multiple detection tool results in increased specificity and sensitivity, also it expands the applications of the diagnostics in developing effective virus disease management strategies to curtail the effects of many of the devastating viral diseases [2, 170]. The accurate diagnostic technique must ensure a reliable assay, which will lead to emplacement of a system of uniformity and quality assurance at a global scale. One of the ways of addressing this issue is by providing diagnostic kits from a common source to stakeholder and researcher across the world. Even though, all these activities require highly skills personnel and experience to optimize and carry out the diagnostic assays in many different environments and interpret results without any ambiguity. With the advancement of molecular techniques, the demand of various detection tools will increase in coming future. The development of protocols with high sensitivity and specificity, rapidness, low cost and feasibility for detection of potato viruses will have apparent impact on the sanitary status of the potato, check on spreading of new or emergent virus in a globalised world. Although, better sensitivity, specificity and simultaneously testing could be attained with new molecular techniques such as microarray, microchip and loop mediated isothermal amplification. Since, many types of viruses affecting potato, a method able to detect several viruses simultaneously would be in demand for testing of planting material, especially for quarantine viruses. Analyses for comparison, validation and standardization are strictly necessary for molecular methods to be accepted and applicability in diagnosis (table -5).

Table 5: Comparison of various types of techniques for diagnostics of potato viruses.

Characteristics	Serological Techniques	PCR based techniques	Hybridization based techniques
Degree of specificity	Often good for viruses	Highly isolate specific	Very high specificity
Level of sensitivity	Less sensitive compare to molecular technique	PCR techniques more sensitive compared to serological	Highly sensitive compare to serological or PCR based technique
Accuracy of the method	Low accuracy but can be adopt in field	Most accurate but in the laboratory	Perfectly accurate but in laboratory
Cost and expertise	Less expensive compared to other molecular methods, less trained personnel required and moderately labour intensive	Moderately expensive, often labor intensive and requires specific instruments, trained personnel required for careful handling of samples and results	Highly expensive but less labor intensive, requires highly specific instrumentation and well trained personnel for careful handling of samples and results
Applicability for rapid detection	Often faster but required a huge amount of samples and typically take days to weeks to complete.	Often time-consuming, often faster and can performed within 1 or 2 days but required less amount of samples	Less time consuming, much faster with high accuracy and can performed within few days, required less amount of samples
Applicability for field work	Field kits are available for most important viruses	Field kits are not available and being developed	Field kits are not available
Speed of detection	Speed is low compared to molecular technique	May require up to 48hrs for reliable results	May require few days for reliable results
Multiplexing	Only one pathogen/virus can detect in a single reaction	Few pathogens/viruses can detect in a single reaction	Simultaneous detection and quantification of thousands of hybridization events
Quantification of inoculums	Quantification capacity is not available	Quantification capacity is available up to multiplexing few pathogens/viruses	Quantification capacity is available up to multiplexing many pathogens/viruses
Robustness and reliability	Less robotic and reliable	Robustness and reliability of PCR based technique is high	Highly robustic and reliable compared to PCR based technique

Furthermore, appropriate sampling protocols as well as sample preparation must be developed and carefully evaluated for each combination of pathogen, plant material and molecular technique. Developing a suitable detection method for a pathogen is an art and a never-ending story, and the concept of accurate detection of viruses, is shifting from conventional methods to advanced molecular techniques targeting multiple approaches [2, 20, 144, 170]. Besides, the serological and PCR based techniques, the access of whole genome sequences and the microarray possibilities, the functional genomics of most potato viruses will soon be determined. This will lead to the recognition of new targets and innovative methods in the diagnostics of potato viruses. Development of RNA microarrays, which enable gene expression analysis of a large number of genes from plant viruses, will provide data for selecting new markers for diagnosis. However, the function of the selected genes will help in host/pathogen interactions. The future will bring more novel tools in the line of genomics to detect potato viruses, based on available new sequences and molecular technologies.

Compliance with ethical standards

Conflict of interest: There is no conflict of interest by the co-authors.

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