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REVIEW

Major In Vitro Techniques for Potato Virus Elimination and Post Eradication Detection Methods. A Review

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Abstract
Potato is an economically important agro-industrial crop that is conventionally propagated, however; its potential transmission of viruses through seed tubers from generation to generation is a major limitation of potato yield production. In order to produce potato virus-free and sufficient amount of potato seed tubers, several approaches of in vitro methods for virus elimination have been developed. Meristem culture has been used alone or combined with techniques such as thermotherapy, electrotherapy, cryotherapy and chemotherapy as the best alternative method for treating potato infected by viruses. Recent literature has shown that to eliminate potato virus significantly depends upon the potato cultivar, antiviral agents, type of virus, the duration of heat treatment. Appropriate duration for efficiency elimination is still under investigation. Viral elimination rate can be detected through serological methods such as enzyme-linked immunosorbent assay (ELISA) and molecular biology technique such as real time reverse transcriptase polymerase chain reaction (real time RT-PCR) that are used for pre and post elimination virus detection to evaluate the efficiency and the accuracy of virus elimination method. The purpose of this review is to highlight virus elimination methods in potato and recommending the most effective tool for virus detection in order to ensure the production of potato plantlet free of viruses.

Resumen
La papa es un cultivo de importancia económica agro-industrial que se propaga de manera convencional, no obstante, la potencial transmisión de virus a través del tubérculo-semilla de generación en generación es una limitación mayor en la producción del rendimiento. Con el fin de producir papa libre de virus y suficiente cantidad de tubérculo-semilla de papa, se han desarrollado varias estrategias de métodos in vitro para la eliminación de los virus. El cultivo de meristemos se ha usado solo o combinado con técnicas tales como termoterapia, electroterapia, crioterapia y quimioterapia, como el mejor método alternativo para tratar papa infectada por virus. La literatura reciente ha demostrado que para eliminar virus significativamente se depende de la variedad de papa, agentes antivirales, tipo de virus, la duración del tratamiento térmico. Aun esta bajo investigación la duración apropiada para la eliminación eficiente. El nivel de la eliminación del virus puede detectarse por métodos serológicos, tales como el inmunoensayo con enzimas conjugadas (ELISA) y técnica de biología molecular, como la reverso-transcripción de reacción en cadena de la polimerasa en tiempo real (RT-PCR) que se usan para la detección del virus pre y post eliminación para evaluar la eficiencia y la precisión del método de eliminación del virus. El propósito de esta revisión es resaltar los métodos de eliminación de virus en papa y en la recomendación de la herramienta más efectiva para la detección de virus para asegurar la producción de plántulas de papa libres de virus.

Keywords Potato seed tubers · Virus elimination methods · Meristem culture · Chemotherapy · Thermotherapy · Electrotherapy · Cryotherapy

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Introduction

Potato (*Solanum tuberosum L.*) plays an important role for sustainable world food security and it is among the four largest crop produced annually at high proportion of the global total area and yield after rice, wheat and

maize (Wang et al. 2011). International center for potato (CIP) and its partners have shown potato as playing a dual role in food security, firstly as cash crop at the market and as food grown for consumption with great nutritive value (Devaux et al. 2014). As demographic growth continues to increase and causes not only steady hunger rates in developing countries, but also uncertainties in crop production yield. It has been highly recommended by FAO (2009), and was found to be one of the primary food sources for andean people (Lutaladio and Castaldi 2009). After being introduced into China as the first potato producing country and among the world biggest population countries through Silk Road, it has made a great contribution in food security of Chinese people (Zhang et al. 2017).

Vegetative propagation using potato tuber seeds are the main used method by farmers (Otroshy 2006). Among the main majors issue associated with vegetative clonal multiplication of potato-seed, susceptibility to viral, bacterial and fungal diseases are accounted (Loebenstein 2001). According to Bamberg et al. (2016), world potato-producing regions are fast infected by viruses and the use of infected plant seed tubers has been reported to be the main avenue of disease spread within pandemic regions (Legg and Thresh 2003). However, farmers are unable to detect visually viral diseases symptoms, due to their variability and poor expression on leaves.

Previous research has reported that about 40 species of viruses are infectious to potato worldwide (Valkonen 2007). The yield reduction due to viral diseases may go up to 75%, and only infection caused by potato virus X (PVX) alone can reduce the yield up to 15–30%; and a high proportion of tuber yield reduction has been reported to be caused by potato leaf roll virus (PLRV) and some strains of potato virus Y (PVY) (Mellor 1987). According to Loebenstein (2001), at least 37 viruses occur worldwide in potato, these are PLRV, potato aucuba mosaic virus (PAMV), potato mop-top virus (PMTV), potato virus A (PV A), potato virus M (PVM), potato virus S (PVS), PVX and PVY to cite a few. And others are found in limited geographical areas of the world; these include arracacha virus B (AVB), beet curly top virus (BCTV), eggplant mottled dwarf virus (EMDV), potato black rings pot virus (PBRV), potato virus U (PVU), potato T virus (PVT) and tobacco streak viruses (TSV).

Among virus species mentioned above, PLRV, PVA, PVM, PVS, PVX, and PVY have been reported to significantly affect cultivated potato and reduce potato crop production in general (Wang et al. 2011). Further results showed PVY to be the most prevalent potato virus worldwide due to some genetic modification that occurred in this viral species (Davie et al. 2017). Example is given to potato tuber necrotic ringspot disease PTNRD which is a most prevalent disease induced by PVY, probably caused by emerging of the biological and genetic diversity of PVY leading to increased viral incidence in seed production (Karasev et al. 2013). Very recently, molecular characterization of potato spindle tuber viroid (PSTVd) isolates from potato has been done to reveal the viroid variants (Qiu et al. 2016). Epidemiologically, the main viruses that are causing mosaics in potato plants are PVY, PVA, and PVM and sometimes by PVX, and these diseases can be caused by one of the viruses or by coinfection of both of them (Kostiw 2011). PVS is prevalent over the world but it is less important given that it only causes little reduction in yield production (Loebenstein 2001).

Effort has been and are still being made to eradicate virus propagation for establishing a national standard and producing certified seed potatoes from in vitro virus-free stock plantlets to certified seed tubers (Grade II) that are delivered to potato growers for commercial production (Danci et al. 2012). The aim of this review is to provide a comprehensive and systematic overview of previous studies related to the developed in vitro methods and modified complex methods for potato virus elimination and post-eradication detection methods.

Major Potato Virus Elimination Methods

Potato is the first major food crop where in vitro techniques have been applied for virus-free plantlets production (Khurana 2004). Apart from the application of genetic engineering techniques for the development of potato virus resistance (Orbegozo et al. 2016), in vitro methods are the most practically used methods to preserve potato genotype as potato cultivars are highly heterozygous clones and are maintained vegetatively (Bamberg et al. 2016). Therefore, to control potato viruses, virus-free seed tubers is an effective and practical means which is used (Faccioli and Colombarini 1996). Several methods have been used to eliminate plants viruses and it has been the same for virus infected potato. Cryotherapy, electrotherapy, meristem culture, thermotherapy, and chemotherapy are the main methods used in many countries (Table 1). Previous literatures have shown thermotherapy and/or chemotherapy to be the most important and reliable methods for obtaining virus-free plants from clonally propagative infected potato (Bamberg et al. 2016), and meristem culture to be the routine method for producing virus-free potato plants (Danci et al. 2012). Cryotherapy was found to be more rapid, efficient and simple method for producing potato virus-free plants than meristem culture (Wang et al. 2006, 2008). All above mentioned methods are discussed in this review.

Table 1 Comparative table of different methods used for potato virus elimination

Methods	Kind of viruses	Combined techniques	Elimination rate in %	Survival rate in %	Referee
Meristem culture	PLRV, PVY		56, 62	50–55	(Wang and Volkonen 2008)
Chemotherapy With 100 mg/L ribavirin	PVM, PVS, PVA	–	100	–	(Yang et al. 2013)
Thermotherapy	PLRV, PVY	Thermotherapy alone	50, 65	75–85	(Waswa et al. 2017)
	PVY	Thermotherapy with Meristem culture		40–50	(Ali et al. 2014)
	PLRV, PVY,PVM, PVS	Thermotherapy combined with chemotherapy	72.7, 71.4, 63.9, 57.4		(Antonova 2017)
Electrotherapy 20 mA/20 min	PLRV/PSTVd		46.72/45.9, 42.85/43.60	Shoot: 84.71 Nodal: 92.35 segments:78.47	(Singh and Kaur 2016)
Cryotherapy	PVS, PVM, PVM + PVS, PLRV, PVY	Cryotherapy combined with meristem culture	38.6, –,0%, 83–86, 91–95	75–85, 91–95	(Wang et al. 2006)
	PVS, PVM, PVM + PVS	Cryotherapy /chemotherapy with ribavirin	100% after 3 subculture		(Kushnarenko et al. 2017)

Standard Virus Elimination Techniques

Meristem Culture for Potato Virus Elimination

The use of meristem culture consists of culturing on a nutrient medium a small (0.1–0.5 mm) piece of tissue removed from the meristematic area. Shoot tips, root tissue are suitable organs for meristem culture, for their high potential of cell division. This technique is considered as a routine method and as the base of standard virus eradication (Sastry and Zitter 2014). Using meristem culture, the production is given within a short period of time and it can be used for any season of the year (Cordeiro 2003). The size of excised meristems, crop cultivar, plant species and virus species are the main factors that often influences success in plant virus elimination (Loebenstein 2001). For potato virus elimination, meristem size determines the proportion of virus elimination, and the rate of obtaining potato virus-free is inversely related to the size of meristem, this is probably explained by the lower virus content within a smaller meristems (Table 2).

Meristem culture technique is more used in many countries to produce potato seed tubers for its capacity to allow rapid cloning and long-term storage of potato plants (Cassells and Long 1982).

However, the fact that the extraction and culture of such a small meristem (0.1 -1 mm) is often problematic (Wang et al. 2008) and can result in non-desirable somaclonal variations, meristem culture is combined with other techniques such as thermotherapy or chemotherapy to enhance the production of potato virus-free plantlets (Cassells and Long 1982; Ali et al. 2014; Aguilar-Camacho et al. 2016).

Chemotherapy Technique to Eradicate Potato Viruses

Plants viruses elimination using antiviral chemicals turns out to be an important technique for the production of virus-free plantlets (Khurana 2004). It is easy and can be easily combined with the routine technique of meristem culture, this greatly helps in elimination of potato virus PVX, PVY, PVM, PLRV (Cassells and Long 1982). Some criteria are required for an antiviral drug to be used in plants chemotherapy (Table 3).

In addition, to be used in clinical chemotherapy, there has been found the effectiveness of antiviral drugs in phyto-viral infection (Sidwell et al. 1972; Panattoni et al. 2013). Literatures have shown ribavirin to be the most promising antiviral chemicals against potato plant viruses (Landesamt et al. 1953). Few results are available in virology and phyto-chemotherapy, this, due to less knowledge of molecular characteristics of many plants viruses and the lack of enough resources within the area compared to clinical virology (Loebenstein 2001). The effectiveness of viral elimination by antiviral agents is proportional to their concentrations and to their form of application (Cordeiro 2003).

Table 2 The correlation between meristem size and virus eradication

The size of meristem	Kind of viruses	Eradication level	Survival and relative growth	References
Meristem with 2 leaves primordia	PLRV, PVY and PVM	100%	N/A	(Al-taleb et al. 2011)
	PVX and PVS	66%	N/A	(Loebenstein 2001)
0 .1mm with or without leaf primordium	PVX and PVS	95%	20%	(Moses et al. 2017)
≤ 0 .3mm	PVA and PVY	85–90%	N/A	(Loebenstein 2001)
	PVX and PVY	Not eliminated	N/A	(Loebenstein 2001)
0.3 mm	PVA and PVY	90%	21%	(Ali et al. 2014)
0 .8mm	PVA and PVY	7%	69%	(Loebenstein 2001)
0 .2mm	PVX	47.4	N/A	(Zaman et al. 2001)
0.4	PVX	21.2	N/A	(Loebenstein 2001)

N/A (Not applicable)

Antiviral Activity of Ribavirin for Eradicating Potato Viruses

To eliminate potato viruses, chemotherapy, especially with ribavirin, was widely applied alone or combined with other methods such as meristem culture, cryotherapy, electrotherapy and cryotherapy (Hu et al. 2015; Singh 2015; Chahardehi et al. 2016; Kushnarenko et al. 2017). Ribavirin, a guanosine synthetic derivative has been synthesized in 1972, and reports have proved it as one of the most promising antiviral drugs (Sidwell et al. 1972; Yang et al. 2013; Singh 2015). Previous studies have shown the difference in the rate of potato virus elimination in single or mixed infections (Table 4). Furthermore, a relatively higher concentration of 75 mg/L to 200 mg/L was used to monitor the change in virus content and to examine the effect of ribavirin on genetic variation within ribavirin-threated plantlets and the results showed a promising effect (Yang et al. 2013). It has been found that PVA, PVM, PVS, and PVY are sensitive to the concentration of 75 mg/L (Loebenstein 2001; Martinelli et al. 2015; Nie and Singh 2015; Kumar 2017). But the same report found PLRV and PVY to be less sensitive to the same concentration, therefore; the conclusion was that more subcultures should be used to improve elimination of PLRV and PVY(Yang et al. 2013). Regeneration which is defined as the percentage of shoot tips with green color 2 weeks after the therapy (Wang et al. 2008) was also monitored during chemotherapy and found that ribavirin has a great regeneration and growth effect (Sastry and Zitter 2014; Yang et al. 2013; Singh 2015). Studies on the influence of chemotherapy with ribavirin found and reported that potato plants regeneration capacity and virus eradication capacity are respectively inversely and directly proportional to the meristems size (Danci et al. 2009). The low regeneration rate with a high concentration is due to the phytotoxic effect of ribavirin (Loebenstein 2001; Agroambientali and Re 2002). However, Danci et al. (2009) and Waswa et al. (2017) has shown that successful eradication of potato viruses seemed to depend upon the duration of treatment and type of cultivar rather than on virus. Research has also showed that, the high concentrations of ribavirin for in vitro potato propagation could successfully eliminate most of the prevalent known potato viruses such as PVA, PVM, PVS, PVX and PVY, but,

Table 3 Some criteria required for the selection of the antiviral agents for plants viruses elimination

Criteria for antiviral agents	Antiviral agents	Concentration mg/l	Types of explants	Kind of viruses	References
Virus elimination capacity	Ribavirin, 2-thiouracil	25	Nodal stem cuttings	PLRV	(Singh 2015)
A great regeneration and growth effect	5-bromouracil			PLRV	(Singh 2015)
Wide broad-spectrum activity against DNA and RNA viruses	Ribavirin, Acyclovir			PVA,PVM, PVS, PVX and PVY	(Sastry and Zitter 2014)
Ability to move systematically in the plant host	Ribavirin	100–150	Shoot tips	PVA, PVM, PVS, PVX	(Yang et al. 2013)
Fulfill the ethic of environmental protection	Ribavirin	100–150	Shoot tips	PVA, PVM, PVS, PVX	(Yang et al. 2013)
Minimal phytotoxic effect to the plant	Ribavirin	100–150	Shoot tips	PVA, PVM, PVS, PVX	(Yang et al. 2013)
Fast eradication effect	Ribavirin and DHT	100–150	Shoot tips	PVA, PVM, PVS, PVX	(Yang et al. 2013)

DHT (2, 4-dioxohexahydro-1, 3, 5-triazine)

Table 4 The difference in the rate of potato virus elimination in single or mixed infections using chemotherapy with different concentrations of ribavirin

Kind of viruses	Ribavirin concentration(mg/L)	Number of culture	Eradication rate (%)	References
PVX	10	Single	80–83	(Klein and Livingston 1982)
PVY	50 with 100 DHT	Single	14–50	(Faccioli and Colalongo 2002)
PLRV	50	Single	15–42	(Faccioli and Colalongo 2002)
PVX,PVS, PVA, PVM	100	Single	High proportion up to 100	(Yang et al. 2013)
PVY	100	Single	0%	(Yang et al. 2013)
PVY	150	Three subcultures	33%	(Yang et al. 2013)
PVY	200	Three subcultures	50%	(Yang et al. 2013)

DHT (2, 4-dioxohexahydro-1, 3, 5-triazine)

indicated PLRV to be more resistant compared to others, therefore, modification might be needed to improve the efficiency of PLRV eradication (Yang et al. 2013; Hu et al. 2015; Chahardehi et al. 2016).

Antiviral Activity of Other Antiviral Reagents for Eradicating Potato Viruses A part from ribavirin used to produce plants virus free, others antivirals reagents have been reported and their effects on plants virus elimination and regeneration have been studied (Dewanti et al. 2016a, b). Effects of different antiviral chemicals including 2-thiouracil on PLRV elimination from In vitro-grown potato was reported and found that ribavirin and 2-thiouracil are effective for the production of potato virus free plantlets (Singh 2015). 2-thiouracil has also been used during virus elimination in plum plants and found that this antiviral drug has elimination capacity on plum pox virus (PPV) and a number of plants can be regenerated during eradication process (AlMaarri et al. 2012; Jakab-Ilyefalvi et al. 2012). Dithioracil can be combined with ribavirin for cymbidium mosaic virus elimination in dendrobium orchid (Katrina and Adelheid 1997). Recently, studies have shown other antiviral reagents such as acyclovir, 5-azacytidine, cytarabine, 5-bromouracil and zidovudine that can be used as an alternative for in vitro production of virus-free potato plants. However, acyclovir was found not to have a strong elimination effect on potato virus as anticipated in early researches on viral elimination drugs (Singh 2015; Dewanti et al. 2016a, b).

Thermotherapy Technique for In Vitro Potato Virus Elimination

Thermotherapy is one of the conventional therapy used to eradicate potato plants viral infection to reduce the concentration of virus for that there is a relationship between temperature and viral RNA silencing (Wang et al. 2008). It has been used against a number of viruses belonging to more than 13 families and an unassigned genus (Panattoni et al. 2013). Thermal sensitivity of some viruses was reported to be lower than that of plant cells and that damage caused to plants tissues by the thermal stress can be more easily reserved than viral damage (Spiegel et al. 1993). The presence of viral RNA in a plant causes the production of the natural antiviral response by the immunity mechanism of an infected plant, with particular reference to virus-induced gene silencing (VIGS) (Ruiz et al. 1998; Carrington et al. 2001; Voinnet 2001). The reports found that VIGS operates ineffectively at low temperature, therefore, the higher the temperature the more the host defense immune system's capacity is enhanced (Panattoni et al. 2013). Further research was done to define temperature factor on antiviral therapy of some plants such as cassava (*Manihot esculenta*) and tobacco (*Nicotiana benthamiana*) plants infected by cassava mosaic disease and similar results were found (Chellappan 2005). So results concluded that there exists a close relationship between temperature and RNA silencing. However, the technique of thermotherapy treatment is potentially effective in eradication of viral particles present in cells, but inefficiency was found with regard to new synthesized viral particles (Panattoni et al. 2013).Thermotherapy method to eliminate potato virus significantly depends upon the potato cultivar, type of virus, and the duration of heat treatment affects plantlet survival (Waswa et al. 2017). The suggestion has been given to further conduct studies to determine appropriate duration for efficient obtaining PVX-free potato plants and this technique can be improved by working in combination with other virus elimination therapies (Cordeiro 2003; Waswa et al. 2017).

Combination of Thermotherapy with Chemotherapy to Enhance the Efficiency of Viral Elimination Potato virus elimination by thermotherapy is selective where it is found ineffective for spherical viruses (Antonova 2017). Reports found chemotherapy as ineffective when a low dose is used (Yang et al. 2013) while inhibiting potato plants development when a high dose is used (Wang et al. 2006). Therefore, it is recommended to combine two therapies to improve the concentration of virus elimination while increasing the regeneration capacity of plantlets. Furthermore, simultaneous use of antiviral agent and temperature, as well as other combinations of viral therapy for potato plants have been reported to ensure effective virus elimination (Chahardehi et al. 2016; Kushnarenko et al. 2017). A significant difference of ($P < 0.05$) has been found between thermotherapy with and without ribavirin for potato plants (Cordeiro 2003), and the same reports showed virus concentration to be potentially eliminated when thermotherapy is combined with chemotherapy with ribavirin. The combination of the two methods is important not only to enhance viral elimination but also to facilitate plantlets regeneration after therapy; an example is given and the great results were found when potato plants are submitted to a combination of chemotherapy with ribavirin and thermotherapy for PVY elimination (Cordeiro 2003). Moreover, the further protocol was proposed for modification of thermotherapy or chemotherapy protocol in order to simultaneously use elevated temperature and ribavirin within a short period of time (Antonova 2017).

Thermotherapy Combined with Meristem Tissue Culture to Enhance Viral Elimination The study has shown a significant influence of temperature on potato therapy during meristem tissue culture technique on both plantlets survival and the percentage of virus eliminated (Panattoni et al. 2013). However, the meristem size, the kind of the virus, the variety of the plant and the duration of the thermotherapy influence the level of heat treatment on the regeneration of meristem derived plantlets and the viral elimination efficiency (Ali et al. 2014). So the combined meristem culture with thermotherapy is better than the use of these two techniques alone. The use of thermotherapy combined with cryotherapy is also another possible complex method of thermotherapy and other therapy, and it has been used for other plants (Wang et al. 2008; Moses et al. 2017).

Cryotherapy Alone or Combined with Other Techniques to Eradicate Viruses

In addition to the above methods, cryotherapy of shoot tip is also a method used to eradicate viruses and a high quantity of samples can be submitted for one turn round. Shoot tips of samples are exposed to liquid nitrogen (−196 °C), and the therapy is found to regenerate a significance virus free plantlets (Wang et al. 2006; Sastry and Zitter 2014). Compared to thermotherapy and meristem culture, this method was found to be the most efficient in term of plantlets survival rate and virus eradication rate (Wang et al. 2006). Not only the efficiency and simplicity of the method but also it is the technique that can be used for the preservation of plantlets for a long time. And it is not a time consuming technique as shown by the previous report (Schäfer-Menuhr et al. 1996). However, the method was found effective for elimination of PVM but ineffective for a mixed infection of PVS and PVM (Kushnarenko et al. 2017). Some cultivars are co-infected by a mixture of viruses such as PVM + PVS + PVY, PVM + PVY or PVS + PVY, and cryotherapy was found inefficient for PVS and PVM eradication but can remove PVY. However, chemotherapy was found inefficient for PVY elimination. Therefore, it was suggested that cryotherapy combined with chemotherapy is much more efficient for co-infected cultivars especially when PVS and PVM are included (Wang et al. 2008; Yang et al. 2013; Kushnarenko et al. 2017).

In Vitro Potato Virus Elimination by Electrotherapy

A part from all the techniques mentioned above, studies have also proven the use of electrotherapy as a simple method that uses electric current while taking plant tissue in any material of infected plant to mitigate virulence through degrading nucleoprotein of the virus (Sastry and Zitter 2014), and this report showed PVX, PVY, PVS, PVA and PLRV to be eliminated by the technique. Compared to the conventional technique of thermotherapy and meristem culture, electrotherapy was found to be the most efficient in term of virus elimination rate and in term of plantlets regeneration (Lozoya-Saldaña et al. 1996). But studies showed that virus structures and plants genotypes respond differently to the technique (Emami et al. 2011). The same data further showed that the intensity of the electric current, the duration of the technique are the factors that influence significantly the capacity of this technique. Singh and Kaur (2016, b) has reported electrotherapy to control PLRV and PSTVd, and prevent the propagation of this dual infection as it is the cause of a serious reduction of the potato yield. During the same study, the use of sprouts, nodal segments and shoot tips were compared to select an appropriate material of the plant for standardizing the technique with a high regeneration rate of the plantlets and a high eradication rate, and was found that shoot tips produce a high rate of potato virus-free plantlets for both PLRV and PSTVd (Fig. 1).

Detection Methods of Potato Virus Eradication

To control potato virus, explants derived from the methods of elimination must be tested for the presence or absence of the

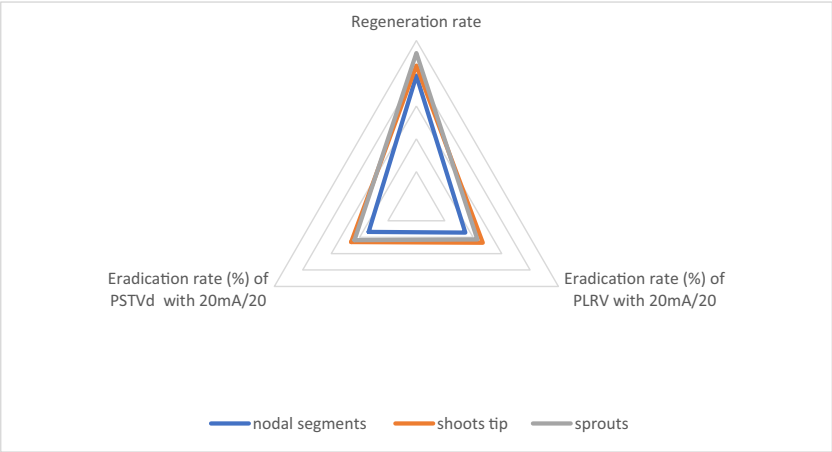

Fig 1 Description of PLRV and PSTVd eradication rate and plants regeneration rate by electrotherapy at 20 mA/20 min with shoot tips, nodal segments, and sprouts. Adapted from Singh and Kaur (2016)

virus. And the early and accurate detection is an essential component of asymptomatic potato virus management, therefore, various highly sensitive techniques, both rapid and non-rapid techniques are used for diagnosis of plant viral diseases (Table 5). Some methods have been conceived; electron microscopy as a rapid test and is also used to determine the shape and the size of the plant virus particles (Loebenstein 2001). For potato virus detection, more sensitive, rapid and cost effective techniques for efficiency detection are very desirable (Wang et al. 2011). ELISA as the routine test method to confirm virus status of meristem cultures based on serological properties (Anjum et al. 2017), nucleic acid based method such as PCR to detect viruses at the molecular level (Lorenzen et al. 2006) have been developed and are frequently used for virus indexing. Dot-immunobinding assay (DIBA), multiplex microsphere immunoassay (MIA), reverse transcription loop-mediated isothermal amplification (RT-LAMP) and next generation sequencing are considered alternative advanced methods but require appropriate expertise for detecting and identifying different potato viruses (Khurana 2004). Speed, specificity, sensitivity, robustness and cost-effective are the major criteria based on designing virus detection techniques (Boonham et al. 2014).

Serological Based Methods for Potato Virus Detection

ELISA, tissue blot immunoassay (TBIA) and quartz crystal microbalance immunosensors (QCMI) have been developed for plants virus detection (Jeong et al. 2014; Kelly et al. 2017). Serological tests have been used for the routine diagnostic protocol. Using ELISA, three majors potato viruses; PVX, PVY and PLRV have been detected, and due to the fact that it is quick, easy to be implemented and to interpret results, is considered the golden standard method for plants virus detection and screening (Jeong et al. 2014). However, the technique is less sensitive compared to molecular biology techniques and lacks the flexibility and compatibility that is inherent in molecular methods (Boonham et al. 2014). As compared to other techniques, it requires a long waiting period of time for post-harvest tubers to be tested, therefore, nucleic acid techniques were developed and are discussed in the following paragraphs (Stammler et al. 1873; Singh et al. 2013).

Development of Molecular Biology Techniques for Potato Virus Detection

Due to the high accuracy and high sensitivity, molecular biology techniques are mostly used in laboratory experiments for potato virus control (Jeong et al. 2014). Compared to serological techniques, molecular biology techniques such as Reverse transcriptase PCR are more reliable, specific, sensitive and more inexpensive (Jeong et al. 2014), and potato viruses PVM, PVS, PVX, PVY and PLRV have been detected using some molecular biology techniques such as, real-time PCR, RT loop-mediated isothermal amplification (LAMP) and Microarray (Oligonucleotide array) (Boonham et al. 2014).

Primers Design for Genetic Materials of Potato Plants Viruses

Specificity of primers and quality of template are two main factors that influence virus detection especially when virus concentration is low (Zhang et al. 2015). Pre-analysis activities are critical and reproducible, therefore, the efficient protocol is a necessity (Martinelli et al. 2015). Reports have been published showing the protocols of genetic materials extraction and primers designing (Nie and Singh 2015; Zhang et al. 2015; Arruabarrena et al. 2016). These reports have shown the use of differential centrifugation followed by RNeasy mini kit (DCR method) to increase the efficiency of RT-PCR technique. To detect potato viruses as for all others kind of plant diseases, the accurate designing of primers is crucial, and this is done using bio-informatics tools such Oligo 7, primer 5, National Center of Biotechnology Information (NCBI) and other online available resources (Martinelli et al. 2015).

Table 5 Popular methods for potato plants virus detection

Methods	Characteristics	Time	Contamination risk	Samples required	Reagents	References
ELISA	Easily interpret, qualitative test	A waiting period of time	Contamination risk	Sprouting of tubers	ELISA reagents	(Nie and Singh 2015)
Conventional RT-PCR		Time reduced	Contamination risk	Sprouting not required	Pre-mix reagents	(Crosslin and Hamlin 2011)
Real-time RT-PCR	Quantitative test, reliable and specific	fast	Low risk of Contamination	Sprouting not required	No agarose gel	(Singh et al. 2013)
Loop-mediated Isothermal amplification	High sensitivity	Short time			Specifically designed loop primers, closed-tube format	(Jeong et al. 2015)

Primers specificity for potato virus detection and identification vary significantly according to laboratories (Crosslin and Hamlin 2011).

Development of Real-Time PCR for Potato Virus Detection

Many techniques of real-time PCR have been developed such as Multiplex RT-PCR and TaqMan real-time RT-PCR (Nie and Singh 2008). These techniques allow virus quantification and monitor the amplification products in real time and do not use electrophoresis for post PCR analysis (Martinelli et al. 2015). Due to the fact that a single potato plant can be infected by several viruses, and given the time and the cost for PCR protocols, the use of Multiplex RT-PCR was found to be a useful technique for it can detect simultaneously two or more sequences in a single reaction (Agindotan et al. 2007). Considering the capability of TaqMan real-time RT-PCR to discriminate small sequence it was found to be the most useful, practicable technique (Singh et al. 2013), and using a quadruplex real-time RT-PCR, PLRV, PVA, PVX, and PVY can be detected simultaneously (Jeong et al. 2015). Optimum dilution, inhibition of RNAses, and the optimization of RT-PCR steps are critical factors that influence the detection of these four viruses from dormant potato tubers (Agindotan et al. 2007). Viruses as for other pathogens are characterized by variation due to mutation of theirs genetic materials; PVY is the typical example for many strains and recombinants types of PVY were recognized (Gao et al. 2014). To differentiate different strains types and mixed infection of this virus a novel test of multiplex PCR assay was developed (Lorenzen et al. 2006).

The Development of Isothermal Amplification for Potato Virus Detection

The need of expensive instrument for PCR analysis that control temperature has pushed scientists to discover DNA polymerase that can amplify DNA at a constant temperature, that technique is called isothermal PCR (Jeong et al. 2014). Among isothermal PCR discovered in plants viruses, the LAMP has been suggested for potato virus detection (Przewodowska et al. 2015) and is performed at a constant temperature for 1 hour using four primers (Almasi et al. 2013). This method has specific importance of increasing sensitivity and reducing turn round time reaction because of its specific designed loop primers that do not require initial template denaturation (Boonham et al. 2014). Furthermore, for field testing, due to its closed-tube format, the LAMP has been found to be able to control substances that can inhibit PCR reaction so that allowing the technique to be used with simple protocols of genetic materials amplification (Kaneko et al. 2007). Ju (2011) showed RT-LAMP as an extremely efficient, sensitive, accurate, simple, fast and reliable detection tool for

PLRV detection within potato tubers. After visual detection of PLRV by a LAMP with the Gene Finder™ dye, it has been an open door for plant pathology in general (Almasi et al. 2013).

Microarray to Detect Potato Viruses without Amplification of Viral RNA

The development of a method that does not require a highly sophisticated machine and special expertise for virus detection was a prerequisite so that a construction of oligonucleotide microarray (oligonucleotide array) has been an answer to this issue (Jeong et al. 2014). Studies have been conducted to detect four potato viruses, PVS, PVY, PVX and PLRV (Bystricka et al. 2005; Boonham et al. 2014). And entirely consistent with standard serological assay results were found (oligonucleotide array) detecting twelve viruses and potato spindle tuber (Maoka et al. 2010; Boonham et al. 2014; Martinelli et al. 2015).The technique was found to be cost effective for simultaneously detecting multiple infections (Agindotan and Perry 2008). The same method has been conducted using a cDNA microarrays and complies with the earlier researches, therefore, it can be used for routine activities such as potato seed certifications (Maoka et al. 2010).

Conclusion and Future Perspectives

Potato viruses are affecting the productivity of potato yield and continue to propagate worldwide. Genomic characterization and sequencing of different types of potato viruses have been done especially for PVY and reveals new strains of these kind of viruses. Therefore, the problem is to find a reliable and efficient method for producing certified potato seed tubers from in vitro virus-free stock plantlets to certified seed tubers (Grade II) that are delivered to potato growers for commercial production and germplasm storage/exchanges. Designing a genetic engineering method that can produce potato viral resistant might be a sustainable solution on one hand. However; on the other hand, the use of genetically modified organisms is still debatable by the international organization of bioethics. Meanwhile, virus eradication using in vitro methods is currently the possible way practically used to preserve potato genotype as potato cultivars are highly vegetative propagated. Plant tissue culture is the most used technique for the production of potato quality-planting material; an especially meristem culture which is a conventional well known method for the production of virus-free plants. However, the fact that the extraction and culture of a small needed meristem tissue is problematic, and can result in non-desirable somaclonal variations, the use of other techniques such as chemotherapy, thermotherapy, cryotherapy and electrotherapy and their improved combinations methods particularly with meristem culture are much more effective for the development of potato virus elimination. Moreover, to detect eradication efficiency, the proposed reliable and accurate serological and molecular biology methods have been conceived to enhance potato virus management. Simplicity, speed, specificity, sensitivity, robustness and cost-effective are the major criteria for selecting detection techniques. All used elimination methods are in general potentially effective for the production of virus-free plantlets, but taking into account plants regeneration rate and viruses eradication, their performance is still poor. Therefore, future perspective for the production of virus-free potato plants are strongly needed. Others methods can be developed such as combined chemotherapy with thermotherapy, as well as the development of modified and complex therapy for eradication of most damaging potato viruses such as the combined thermotherapy and cryotherapy for efficient potato virus elimination.

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