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Plant Viral Disease Management in the Genomics Era

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ABSTRACT

Viral diseases pose a significant threat to crop production and quality. This is particularly true for crops in the tropical and subtropical regions and those whose propagation relies on vegetative tissues. Effective management of viruses and their vectors thus play a pivotal role in crop production. Various practices such as stringent phytosanitary measures, seed certification programs and vector controls have been widely applied in various crops. As more and more viruses, viroids included, have been fully sequenced and their transmission modes better understood, molecular detection/diagnosis/monitoring of target viruses/viroids in plants and vectors have been gradually development and employed. Modern technologies such as polymerase chain reaction (PCR)-based and nucleic acid hybridization-based techniques including PCR, real-time PCR and microarray demonstrate a great potential for efficient and accurate detection of a large number of viruses/viroids as well as other formats of pathogens in real-time, thus providing accurate guidance for controlling/managing the pathogens. Meanwhile, the genomic era technologies have also significantly expanded our understanding of the molecular basis of host-virus-vector interactions, which will undoubtedly improve virus disease management in various aspects including more efficient development and utilization of resistant cultivars especially those with multiple resistances and more efficient management of vectors and vector-mediated transmission.

Keywords: emerging virus, molecular diagnostics, potato, certification, resistance

CONTENTS

INTRODUCTION

Plant viruses cause significant agricultural losses worldwide, especially in tropical and subtropical ecosystems, and in crops that are vegetatively propagated. Although the exact yield reductions and the economic losses due to virus infection are highly varied, depending on the nature of the virus and the crop species and/or cultivars, a total failure of a particular crop due to virus infection is not impossible (Waterworth and Hadidi 1998). For example, it has been estimated that viruses of the genus *Tospovirus*, family *Bunyaviridae*, cause a total loss of more than one billion dollars per year; and viruses of the genus *Begomovirus*, family *Geminiviridae*, result in the loss of millions of tons of food annually (Harrison and Robinson 1999). Members of the genus *Potyvirus* in the family *Potyviridae*, including *Potato virus Y* (PVY), *Plum pox virus* (PPV), *Soybean mosaic virus* (SMV) and *Bean common mosaic virus* can cause up to 100% yield losses in their respective host crops (Shukla *et al*. 1994). Nevertheless, many viruses exert their effects on crop plants in a variety of more subtle ways such as reduction in vigour and growth. Many members of the genus *Carlavirus* in family *Flexiviridae* are examples. *Potato virus S* (PVS), which occurs worldwide in the potato crop, induces little or no symptoms on most potato varieties yet causes up to 20% yield reductions (Wangai and Lelgut 2004). Therefore, effective management of viruses plays a pivotal role in crop production to minimize crop yield losses and maximize economic returns.

Virus disease control relies on integration of germplasm resources, technologies, and production practices. All of these are reflected in two fundamental aspects: virus avoidance and virus resistance. Potato seed certification system, virus vector control, and virus infection prevention are the primary components in virus avoidance. Developments of cultivars bearing virus resistance genes and disruptions of virus establishment in host plants through virus-transgene or interference RNA are the main focuses in virus resistance.

This mini-review will discuss various perspectives of plant virus management in the genomics era, including current challenges in plant virus disease control, molecular virus diagnostics, virus vector management, and resistance. In much of the review, potato crop will be used as a model for virus disease management.

CHALLENGES IN PLANT VIRUS MANAGEMENT IN THE ERA

Knowing what the crops might face is the key to protecting them from detrimental pathogens. Rapid and accurate identification of viruses, viroids included, is thus essential in plant virus management. This has become particularly important given the fact that new viruses/virus strains are emerging due to various factors such as global climate

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change, international trade in agricultural and horticultural produce, and the exchange of materials such as germplasm.

Changes in climate could have a significant indirect influence on patterns of virus spread, either through changes in cropping patterns or changes in the distribution of virus vectors (Boonham *et al*. 2007), especially insect-vectors such as *Thrips palmi* (Mumford *et al*. 1996; McDonald *et al*. 1999). Globalization and the development of international trade in agricultural and horticultural produce, which is breaking down the traditional geographical barriers to the movement of pathogens is probably one of the biggest challenges. The potential for the importation of nonindigenous plant viruses and virus vectors has grown significantly. Whiteflies are effective vectors for at least 50 geminiviruses (Bedford *et al*. 1993) including *Cotton leaf crumple virus*, *Chino del tomate virus*, *Lettuce infectious yellows virus* and watermelon curly mottle strain of *Squash leaf curl virus* (Brown and Nelson 1984; 1986; 1988). In various parts of China, B and Q-biotypes of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), which possess various insecticide-resistant capacities, have been detected (Ma *et al*. 2007). These insects were traced back to an International Horticultural Exposition held in 1999 in Kunming City, Yunnan Province, where large quantities of poinsettias and other ornamentals were imported to China from more than 60 countries (Zhang *et al*. 2005). The soybean aphid (*Aphis glycines*), which is native to Asia, was first discovered in North America in 2000 and has now been found in at least 30 states in the USA and three provinces in Canada (Ragsdale *et al*. 2011). The soybean aphid is capable of transmitting many viruses including SMV, *Alfalfa mosaic virus* and *Tobacco ringspot virus* on soybean plants (Clark and Perry 2002) and PVY and *Potato leafroll virus* (PLRV) on potato plants (Davis *et al*. 2005; Davis and Radcliffe 2008). It has thus been implicated in spread of PVY in potato crops in certain regions in the USA (Davis *et al*. 2005; Davis and Radcliffe 2008). The introduction of *Plum pox virus* into North America is another example. PPV causes plum pox or Sharka disease in *Prunus* species including plum, peach, apricot, nectarine, sweet cherry, and sour cherry and is thus considered the most devastating disease of stone fruits (Roy and Smith 1994). The virus was established in Europe approximately 40 years ago (Roy and Smith 1994), and it has been identified in both the United States and Canada in the past 10 years (Levy *et al*. 2000; Thompson *et al*. 2001). The establishment of this virus could have massive repercussions on the continent's stone fruit industry. In order to try and prevent this, both countries are pursuing PPV eradication programs (James *et al*. 2003; Damsteegt *et al*. 2007).

The occurrence and spread of the potato tuber necrosis strain of PVY, i.e., PVY^{NTN} could be considered another example. Unlike the common strain (PVY^{O}) and the tobacco veinal necrosis strain (PVY^{N}), PVY^{NTN} induces potato tuber necrotic ringspot disease (PTNRD) in sensitive potato varieties and veinal necrosis in tobacco plants (**Fig. 1**), thus posing a significant threat to the potato industry (Singh *et al*. 2008). PVY^{NTN} was first found in Hungary in the 1980s (Beczner *et al*. 1984) and subsequently in many other parts of the world (Singh *et al*. 2008), suggesting that the spread might be a recent event. Capable of causing deformed-tubers, *Potato mop-top virus* (PMTV), a fungus (*Spongospora subterranea*)-transmitted soil-borne RNA virus, was not known to be in North America until 2003 (Lambert *et al*. 2003; Xu *et al*. 2004), strongly suggesting a recent introduction to the continent.

Ornamental plants also present additional risks due to the sheer diversity of species and families involved (Boonham *et al*. 2007). Ornamental plants have been shown to be rich viroid reservoirs (Bostan *et al*. 2004; Nie *et al*. 2005), and regarded as the "silent carrier of evolving viroids" (Singh and Teixeira da Silva 2006). Indeed, economically important viroids such as *Citrus exocortis viroid*, *Tomato chlorotic dwarf viroid* (TCDVd) and *Potato spindle tuber viroid* (PSTVd) have been found in ornamental species (Bostan *et al*. 2004; EPPO 2006; Singh and Dilworth 2008).

Fig. 1 *Potato virus Y* tuber necrosis strain (PVY^{NTN}) induced symptoms in tobacco plant and potato tubers. Left, tobacco cv. "Samson". Right, tubers of potato cv. "Norchip" at 1 (top) and 3 (bottom) months post-harvest and "Ranger Russet" at 3 months post-harvest (adopted from Nie and Singh 2003b).

It is particular noteworthy that TCDVd, which is seed-transmissible in tomato and could survive under subzero conditions in ornamental hosts (Singh and Dilworth 2009; Singh *et al*. 2009), has been reported in the greenhouse tomato crops in many countries including Canada, UK, USA and Japan (Singh *et al*. 1999; James *et al*. 2008; Matsushita *et al*. 2008; Ling *et al*. 2009), and has caused significant yield and economic losses.

RAPID VIRUS DIAGNOSTICS AND STRAIN DETERMINATION

Molecular diagnostic technologies have played an important role in plant virus detection and strain differentiation in many different crops and cropping systems (Martin *et al*. 2000). Unlike the traditional diagnostic tools including the indicator plant-based bioassay, which are extremely time consuming, and the antibody-based enzyme-linked immunosorbert assay (ELISA), which requires specific and quality antibodies, molecular diagnostics is fast, flexible and accurate. Moreover, it can significantly increase the diagnostic efficiency by simultaneous detection of multiple viruses or viral samples (Martin *et al*. 2000; Hadidi *et al*. 2004; Boonham *et al*. 2007). It is particularly effective for detection of viroids (Diener 1991) due to the fact that viroids possess a single-stranded circular non-protein-encoding RNA genome and can not be detected by current immunology technology like regular ELISA (Fonseca *et al*. 1996). Nevertheless, a combination of diagnostic methods including ELISA, bioassay and nucleic acid-based procedures is needed to better understand and characterize a particular virus or virus strain, as evidenced in cases involving PVY^{NTN} (Mehle *et al*. 2004; Hu *et al*. 2009) and $PVY^{N:O}$ (Singh *et al*. 2003; Nie *et al*. 2004).

Molecular virus diagnostic methodologies include nucleic acid hybridization (NAH) and DNA amplification. The NAH methods include Northern and Southern blots, dot-blots and micro-/macroarrays. The DNA amplification consists of polymerase chain reaction [PCR, including reverse transcription (RT)-PCR], real-time PCR (including real-time RT-PCR), and isothermal amplification of nucleic acids. NAH was the first molecular virus diagnostic technique used in plant virology (Gould and Symons 1983). Although varied in sample preparation and equipment requirement, all NAH formats involve virus-specific probes that are complementary to the virus genome to hybridize with the nucleic acid of the target virus, and the signals of the labelled probes or the targeted nucleic acids are thereafter detected.

Probably the simplest of all molecular methods used for virus/viroid detection, dot-blot hybridization was the first NAH being developed and is still used by diagnosticians. More recently, reverse dot-blot hybridization has been developed (Kawasaki *et al*. 1993) and employed to detect plant viruses (Hsu *et al*. 2005). In both dot-blot and reverse dot blot hybridization assays, the nucleic acids or virus/viroid-specific probes are applied and immobilized on various spots of a nylon or nitrocellulose membrane. The bound

sequences thereafter hybridize with radioisotope or non-radioisotope molecule (e.g. digoxigenin and biotin)-labelled probes or nucleic acids (Singh and Nie 2001; Hsu *et al*. 2005). In the potato crop, dot-blot assays have been used successfully for detecting various viruses/viroids from different tissues including dormant potato tubers (Welnicki and Hiruki 1992) and leaves (Loebenstein *et al*. 1997); whereas reverse dot-blot hybridization assays have been employed to detect various potyviruses including PVY (Hsu *et al*. 2005).

DNA array technology is the most recent development in NAH-based virus diagnosis (Wang *et al*. 2002; Boonham *et al*. 2007). The technology was first described in 1995 for quantitative analysis of the expression of multiple genes simultaneously (Shena *et al*. 1995), and it has since been adopted for various areas including genomics, biomedical related researches, and pathogen diagnosis. It has the potential for the simultaneous detection of a large number of pathogens including fungi, bacteria, and viruses/viroids (Matin *et al*. 2000; Hadidi *et al*. 2004; Boonham *et al*. 2007). Two types of arrays, namely macroarrays and microarrays, have been used (Hadidi *et al*. 2004; Agindotan and Perry 2008). The two virtually differ only in the sample spot sizes and the number of spots on the support. Macroassays contain spot sizes of about 300 μm whereas the sizes of the sample spots in microarrays are less than 200 μm in diameter and the latter usually contain thousands of spots (Hadidi *et al*. 2004). In potato, both the nylon membrane-based macroarrays and the glass-slide-based microarrays have been used successfully to detect different viruses including *Potato virus X* (PVX), PVY, *Potato virus A* (PVA), PMTV, PLRV, PVS and PSTVd (Bystricka *et al*. 2003; Boonham *et al*. 2003; Bystricka *et al*. 2005; Agindotan and Perry 2007, 2008; Maoka *et al*. 2010). Employing cDNA probes, Boonham *et al*. (2003) successfully differentiated two closely related PVS strains, PVS-O and PVS-A using microarray, even though the same array failed to discriminate PVY^{O} from PVY^{N} and PVY^{NTN}. The sensitivity in this microarray assay was comparable with ELISA (Boonham *et al*. 2003). However, the sensitivity can be improved if virus- or strain-specific single-stranded oligonucleotides are used as the captured reference DNA fragments (Bystricka *et al*. 2005). Indeed, a microarray test using the oligo-probes not only detected the targeted viruses (PVA, PVS, PVM, PVY and PLRV), but also differentiated the closely related strains of PVY, i.e., PVY^{O} and PVY^{NTN} (Bystricka *et al*. 2005). Both oligo- and cDNA probes have also been employed in macroarrays for detections of potato viruses and viroid (Agindotan and Perry 2007, 2008; Maoka *et al*. 2010). It is noteworthy that the macroarray results were completely consistent with those obtained using ELISA when applied to potato field isolates (Agindotan and Perry 2008). Moreover, the sensitivity of macroarray can be significantly enhanced when involving PCR amplification and biotin labelling of the target cDNAs (Maoka *et al*. 2010). This macroarray assay was 5×10^2 to 4 x 10^6 times more sensitive than ELISA and 5 to 5×10^6 times more sensitive than RT-PCR assay (Maoka *et al*. 2010). Although the feasibility, especially the requirements for highly specific equipment and skilled personnel as well the cost, needs to be evaluated further, the array technology will likely be used more widely in various areas such as in plant virology research as well as certification and quarantine programs.

DNA amplification-based disease diagnosis began in the mid 1980s using polymerase chain reaction (Saiki *et al*. 1985). Since then, the approach has been widely used for disease diagnosis and pathogen detection (Henson and French 1993; Hadidi *et al*. 1995). PCR enables exponential amplification of specific DNA sequences *in vitro*, thus providing extremely high sensitivity and accuracy in disease diagnosis and pathogen detection. Since viroids and many viruses possess RNA genomes, reverse transcription (RT) is needed for successful PCR amplification of the target RNA segments. Simplex RT-PCR, in which only one pair of virus-specific primers is present, has been successfully applied for detection of potato viruses/viroids from different plant tissues such as dormant tubers, stems, sprouts and leaves as well as single aphids (Singh and Nie 2003). It is particularly noteworthy that RT-PCR has been successfully used to monitor PLRV and PVY in growing tubers during the growing season, thus providing an accurate early forecast prior to the harvest (Singh *et al*. 2003b).

Multiplex PCR/RT-PCR, which accommodates several pairs of primers in a single reaction in contrast to several individual PCR reactions, can lead to a considerable savings of both time and efforts as well as supplies (Singh and Nie 2003). Multiplex PCR/RT-PCR is particularly useful in crops like potato due to the frequent occurrence of mixed-infections (Boonham *et al*. 2002; Singh and Nie 2003). Multiplex PCR, especially multiplex RT-PCR, can be affected by many factors. Optimization of the reaction by adjusting various parameters such as the concentrations of Mg^{2+}, dNTPs, and primers are also needed (Singh and Nie 2003). Moreover, the cDNA quality can significantly impact subsequent PCR reactions. Two primer systems, namely the oligo(dT) and the random hexonucleotides, have been used for cDNA synthesis for common potato viruses/viroid including PVY, PVA, PVS, PVX, PLRV and PSTVd (Nie and Singh 2000, 2001). Except PLRV and PSTVd, most potato viruses possess an mRNA-like RNA genome. Therefore, oligo(dT) can be used as the common primer to synthesize cDNAs of the polyadenylated viruses (Nie and Singh 2000). Random hexonucleotides, which contain 4^6 different nucleotide combinations, exhibit their suitability for synthesis of cDNAs for both polyadenylated and non-polyadenylated viruses/viroids (Nie and Singh 2001), suitable for the subsequent multiplex PCR detection of the pathogens. Multiplex RT-PCR procedures are also very useful for detection and differentiation of various strains/substrains of PVY (Nie and Singh 2002, 2003a; Nie *et al*. 2004; Piche *et al*. 2004; Crosslin *et al*. 2005; Hu *et al*. 2009; Chick Ali *et al*. 2010a; Nie *et al*. 2011). The P1 protein is the least conserved protein among the potyviruses (Domier *et al*. 1987) and various strains of PVY (Tordo *et al*. 1995; Nie and Singh 2002a). Based on the sequence homologies among various PVY strains/substrains including Eu-$PVY^{N/NTN}$, NA-$PVY^{N/NTN}$, and PVY^{O}, a multiplex RT-PCR using one common reverse primer plus several strain-specific sense primers has been developed, enabling simultaneous differentiation of those isolates (Nie and Singh 2003a; Piche *et al*. 2004). The unique genome structure of the recombinant isolates such as $PVY^{N:O}$, Eu-PVY^{NTN} (Nie and Singh 2003a), PVY^{NTN}-HN2 or $PVY^{NTN\text{-}NW}$ (Hu *et al*. 2009; Chikh Ali *et al.* 2010b) (**Fig. 2**) provides an opportunity to design primers targeting the recombinant joints (RJ), enabling the unequivocal discrimination of these closely related groups (**Fig. 3**). It is reasonable to predict that more formats of multiplex RT-PCR will be developed as more information regarding the molecular biology and pathology of a given virus or virus strain becomes available.

Real-time PCR is a recent technology being adopted for detection of various plant viruses/viroids (Boonham *et al*. 2004, 2005; Balme-Sinibaldi *et al*. 2006). This technology is based on one of the two principles for real-time monitoring of the amplification products: incorporation of fluorescence dyes such as SybrGreen® into the newly synthesized double-stranded DNA (Ririe *et al*. 1997) or degradation of a fluorescent dye-labeled probe (TaqMan®, Molecular beacon® or Scorpio®) during the polymerization of new strands (Mackay *et al*. 2002; Schena *et al*. 2004). In the potato crop, real-time RT-PCR has been developed to simultaneously detect multiple viruses including PLRV, PVA, PVX and PVY in a two-step assay (Agindotan *et al*. 2007); and PLRV, PVX, PVS and *Tomato spotted wilt virus* (TSWV) in a single-tube assay (Mortimer-Jones *et al*. 2009). Moreover, a TaqMan®-based real-time quantitative RT-PCR has also been developed to detect PVY^{O} and PVY^{N} based on the single nucleotide polymorphism at A/G_{2213} of PVY genome (Jacquot *et al*. 2005; Balme-Sinibaldi *et al*. 2006).

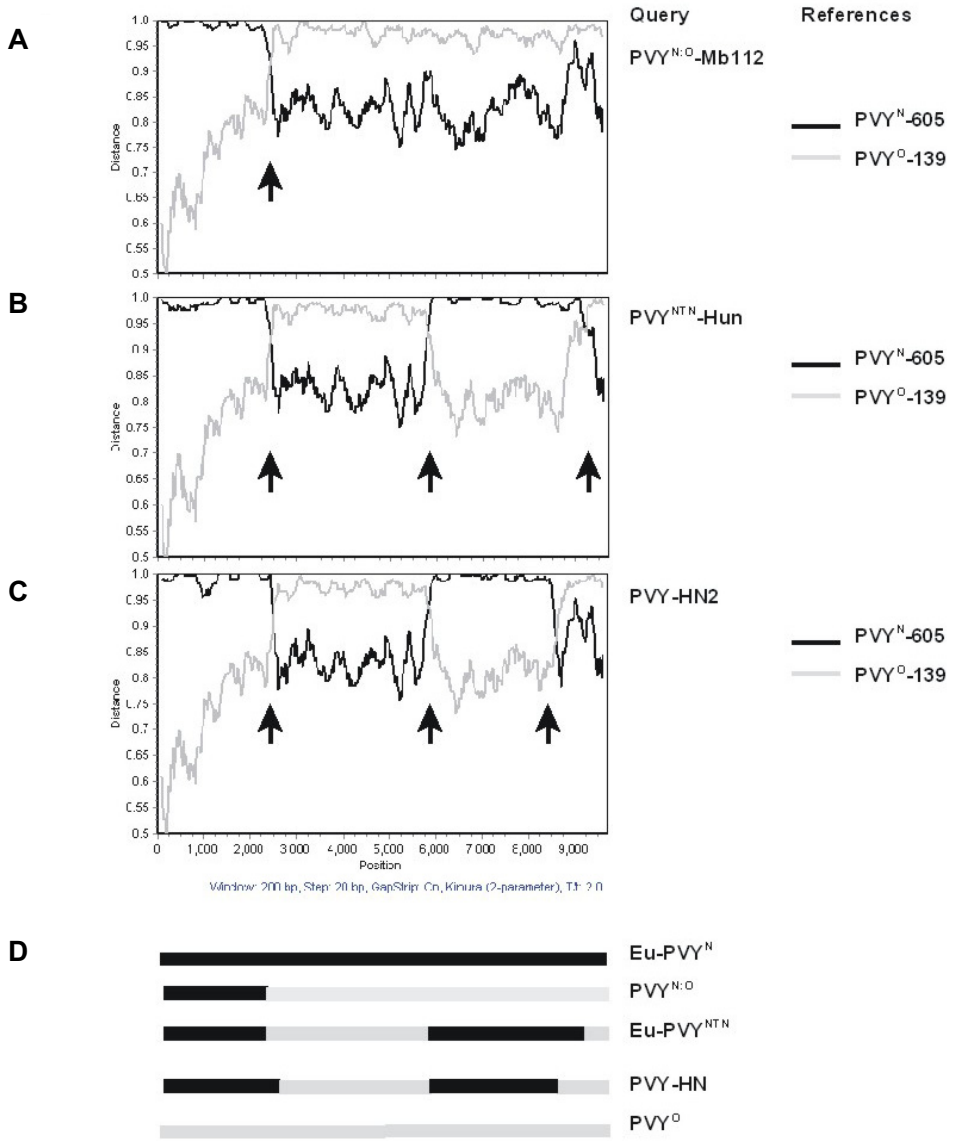

Fig. 2 Analysis of genome structure of *Potato virus Y* (PVY) for putative genome recombination. (**A** to **C**) Analysis of nucleotide sequences of PVY. (**A**) $PVY^{N:O}$-Mb112; (**B**) PVY^{NTN}-Hun; and (**C**) PVY^{NTN}-HN2 (Query). The accession numbers of the isolates are: PVY^{O}-139, U09509; PVY^{N}-605, X97895; PVY^{NTN}-Hun, M95491; $PVY^{N:O}$-Mb112, AY745491; PVY-HN2, GQ200836. (**D**) Schematic diagram of PVY genome structure based on A to C (Hu *et al*. 2009).

The procedures not only distinguish the two pathotypically different PVY isolates but also simultaneously quantify the isolates (Balme-Sinibaldi *et al*. 2006). The technology has also been employed to estimate the PPV RNA targets acquired and transmitted by single aphids (Olmos *et al*. 2005; Moreno *et al*. 2009), thus enabling the quantitative assessment of the transmission efficiency of the virus and its epidemiology.

Loop-mediated isothermal amplification of DNA (LAMP) technology offers an alternative approach for efficient and cost-effective amplification of target DNA molecules (Notomi *et al*. 2000). Using four specially designed primers that include six short sequences matching the target DNA fragment, the DNA is amplified under isothermal conditions (65°C) within a short period of time. The reactions are easily monitored by detecting the turbidity caused by the formation of magnesium pyrophosphate (Mori *et al*. 2001). The technology has been adopted to detect various plant viruses/viroids including *Japanese yam mosaic potyvirus* (Fukuta *et al*. 2003), TSWV (Fukuta *et al*. 2004), *Tomato yellow leaf curl virus* (Fukuta *et al*. 2003), PVY (Nie 2005), PPV (Varga and James 2006), and *Peach latent mosaic viroid* (Boubourakas *et al*. 2009). In combination with a real-time turbidimeter that monitors the turbidity (Boubourakas *et al*. 2009) or a real-time thermal cycler that measures fluorescence derived from an intercalation dye (Aoi *et al*. 2006), real-time LAMP/RT-LAMP assays can be achieved.

CERTIFICATION AND VECTOR MANAGEMENT FOR VIRAL DISEASE CONTROL: POTATO AS A MODEL CROP

Potato (*Solanum tuberosum* L.) is the world's fourth most important crop following rice, wheat, and corn. As a vegetatively propagated crop, potato is particularly prone to virus diseases. Seed potato certification programs, which play a central role in virus and virus disease control in many countries including Canada (Shepard and Clatfin 1975; Slack and Singh 1998; Love *et al*. 2003), will continue to be the most important component in virus disease management in the genomics era. With the wider acceptance of various molecular diagnostic tools, it is expected that the technologies will be integrated into routine post-harvest test systems in certification laboratories/agencies.

Many viruses need insect vectors such as aphids to transmit; some need fungi and nematodes to spread; and others simply use mechanical contacts for transmission. Aphids are by far the most important vectors, responsible for the transmission of various viruses including PVY,

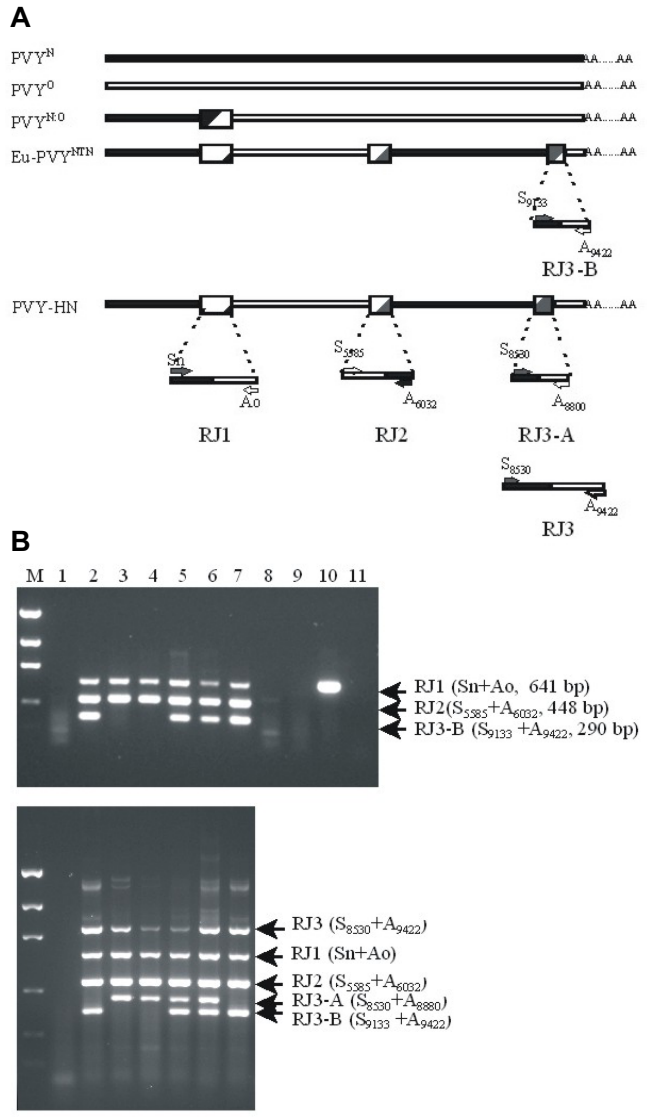

Fig. 3 Multiplex RT-PCR detection of recombinant PVY isolates. (**A**) Schematic genome structure of PVY^{O}, PVY^{N}, $PVY^{N:O}$ and recombinant PVY^{NTN} (PVY^{NTN}-Hun and PVY^{NTN}-HN2) as well as primer locations and resulted PCR products. (**B**) Multiplex RT-PCR detection for recombinant joints. Top panel: Triplex RT-PCR using primers for RJ1, RJ2 and RJ3-B; Bottom panel: Tetraplex RT-PCR using primers for all RJs. Lanes 1, Healthy; lanes 2 and 7, Eu-PVY^{NTN}; lanes 3-4, PVY^{NTN}-HN2; lanes 5-6, Eu-PVY^{NTN} + PVY^{NTN}-HN2; lanes 8-11, PVY^{N}, PVY^{O}, $PVY^{N:O}$ and NA (non-recombinant)-PVY^{NTN}, respectively (adopted from Hu *et al*. 2009).

PLRV and PVA. Therefore, effective management of aphids is one of the most important parameters in controlling potato viruses. PLRV can be transmitted by several species including green peach aphid (*Myzus persicae*), potato aphid (*Macrosiphum euphorbiae*), and buckthorn aphid (*Aphis nasturtiti*) in a circulative persistent manner; whereas other known aphid-transmitted potato viruses including PVY are transmitted through a nonpersistent attachment-detachment mechanism (Radcliffe and Ragsdale 2002; Harrison and Robinson 2005). Due to the nonpersistent transmission mode, PVY can be transmitted by more than 50 species of aphids, including some species that cannot colonize in potato (Radcliffe and Ragsdale 2002; Pelletier *et al*. 2008). Although not as effective as green peach aphids, soybean aphids and grain aphids (e.g., bird-cherry-oat aphids, *Rhopalosiphum padi*) have been suggested to play a significant role in the spread of PVY in certain regions during certain years (DiFonzo *et al*. 1997; Davis *et al*. 2005; Pellieter *et al*. 2008). Monitoring of aphid behavior, migration, and population dynamics is an important part in control of aphid-transmitted viruses (Thomas *et al*. 1997). Moreover, assessment of potential risk of virus spread through indexing viruliferous aphids by molecular diagnostic methodologies can provide more precise guidance for appropriate action such as mineral oil application, insecticide application and/or crop top-killing (Singh and Nie 2003). RT-PCR has been applied to detect PLRV and PVY from composite and single aphids (Singh *et al*. 1996). This technology as well as other nucleic acid-based procedures such as real-time RT-PCR and micro-/macro-arrays may play a more important role in management of aphids and aphid-transmitted viruses in the future.

VIRUS RESISTANCE

Use of resistant cultivars is probably the most cost-effective and reliable approach for control of virus diseases (Solomon-Blackburn and Barker 2001a, 2001b; Kang *et al*. 2005). In potatoes, two types of resistance, i.e., hypersensitive resistance (HR) and extreme resistance (ER), have been recognized (Gebhardt and Valkonen 2001; Solomon-Blackburn and Barker 2001a; Kang *et al*. 2005). Two resistance genes, i.e., *Rx-1* and *Rx-2* against PVX have been isolated (Bendahmane *et al*. 1999, 2000), and several *R* genes including *Ry* genes (Ry_{adg}, Ry_{sto}, $Ry\text{-}f_{sto}$) to PVY (Brigneti *et al*. 1997; Kasai *et al*. 2000; Flis *et al*. 2005), *Ns* to PVS (Marczewski *et al*. 2002), and Ra_{adg} to PVA (Hämäläinen *et al*. 1998) have been mapped. As an increasing number of molecular markers of the *R* genes become available, marker-based selection of progenies with desired virus resistance is anticipated to play an important role in potato breeding programs. As the complete genome sequence of potato becomes available (www.potatogenome.net), various genomics tools such as high resolution DNA melting analysis on markers (De Koeyer *et al*. 2010) will accelerate the selection of lines with multiple virus resistance. An alternative strategy for developing virus resistance is through the virus-derived transgene. This might be particularly useful when genetic sources of host resistance are scarce, e.g., papaya to *Papaya ringspot virus* (Gonsalves 1998), or where the resistance is controlled by polygenes, e.g., the resistance against PLRV in potato (Marczewski *et al*. 2005). Transformation with nonpathogen-derived sequences such as the cloned host resistance genes can also lead to an elevated resistance against a particular virus or a broad-spectrum of viruses (Solomon-Blackburn and Barker 2001b). The transgene-derived virus resistance holds the promise for control of virus disesases in many different crops.

CONCLUDING REMARKS

Significant progress has been made in last twenty years in the study of plant viruses/viroids, in terms of understanding their molecular and biological properties as well as interactions with vectors and hosts. This wealth of knowledge, combined with various cutting-edge technologies, has led to numerous molecular diagnostic tools and novel strategies for rapid and accurate detection and effective management of the pathogens. Despite the advances, virus and virus disease control remain to be challenging due to various factors such as global climate change, international trade and plant materials exchange, leading to emerging/re-emerging new viruses/viroids or virus strains. In case of the potato crop, seed potato certification as well as effective management of aphid vectors will continue to play an essential role in virus disease management. In addition, understanding, development and utilization of various resistant resources including host and virus-derived resistances will be significantly accelerated in the genomics era.

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