식물병연구

Res. Plant Dis. 20(3) : 173-181(2014)

Mini-Review | Open Access

http://dx.doi.org/10.5423/RPD.2014.20.3.173

A Review of Detection Methods for the Plant Viruses

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Received July 31, 2014
Revised September 2, 2014
Accepted September 4, 2014

The early and accurate detection of plant viruses is an essential component to control those. Because the globalization of trade by free trade agreement (FTA) and the rapid climate change promote the country-to-country transfer of viruses and their hosts and vectors, diagnosis of viral diseases is getting more important. Because symptoms of viral diseases are not distinct with great variety and are confused with those of abiotic stresses, symptomatic diagnosis may not be appropriate. From the last three decades, enzyme-linked immunosorbent assays (ELISAs), developed based on serological principle, have been widely used. However, ELISAs to detect plant viruses decrease due to some limitations such as availability of antibody for target virus, cost to produce antibody, requirement of large volume of sample, and time to complete ELISAs. Many advanced techniques allow overcoming demerits of ELISAs. Since the polymerase chain reaction (PCR) developed as a technique to amplify target DNA, PCR evolved to many variants with greater sensitivity than ELISAs. Many systems of plant virus detection are reviewed here, which includes immunological-based detection system, PCR techniques, and hybridization-based methods such as microarray. Some of techniques have been used in practical, while some are still under developing to get the level of confidence for actual use.

Keywords : ELISA, PCR, Plant Virus Detection, Symptomatic diagnosis

Introduction

Generally speaking, viruses are very tiny compared to other groups of plant pathogens like fungi and bacteria which can be visualized through microscopes but plant viruses are too small to observe using light microscopes and they can be seen only using a transmission electron microscope and are made of a coat protein and a types of nucleic acid, DNA or RNA based on the nucleic acid core carrying genetic information (Ellis *et al*., 2008). Since *Tobacco mosaic virus* (TMV) was first recognized over a century ago, more than 1000 of plant viruses have been found (King *et al*., 2011; Scholthof, 2000). It has been known that like other plant pathogens including bacteria, fungi, and phytoplasma, plant viruses spread and cause major economic losses to many crops such as barley, corn, potato, rice, and wheat (Agrios, 2005; Ellis *et al*., 2008; Strange, 2005). Virus is ranked as the second most important plant pathogens following fungi (Vidaver and Lambrecht, 2004). Economic loss has been estimated more than several billions dollars per year worldwide because of plant viruses (Hull, 2002; Plant Viruses, 2003). Plant viruses cause the damage inside plant cells by intervening the allocation of resources that the plant has produced through photosynthesis.

The crop damages owing to viral diseases are difficult to predict, because it depends on region, virus strain, host plant cultivar/variety, and time of infection (Strange, 2005). Symptoms of viral diseases include crinkling, browning of leaf tissues, mosaic, and necrosis. Sometimes, however, symptoms may not be visually detected because infection of plant viruses causes no symptoms (Bove *et al*., 1988; van der Want and Dijkstra, 2006). In addition, plants can also display virus-like symptoms when plants respond to unfavorable weather, nutritional imbalances, infection by other types of pathogens mentioned above, damage caused by pests or abiotic agents and others (van der Want and Dijkstra, 2006). Thus, viral disease diagnosis by symptoms is more difficult than other pathogens (Lievens *et al*., 2005).

The diagnosis is the basis to manage plant diseases and to predict the crop loss by infection of plant pathogens (van der Want and Dijkstra, 2006). Accurate diagnosis of virus diseases, is the first important step for crop management system (Aboul-Ata *et al*., 2011). Since after virus infection, agrochemical treatments

to plants do not lead to an effective control, viral diseases most effectively managed as control measures are applied before infection (Aboul-Ata *et al*., 2011). In order to prevent plant viral diseases, it is important to figure out the causes and to distinguish diseased plants and unaffected plants that show virus–like symptoms (Pearson *et al*., 2006).

As the internationalization of the domestic agricultural market, virus diagnostics is very essential to use high-quality seed as well as virus-free seeds (Lievens *et al*., 2005; Wang *et al*., 2011). As mentioned above, unlike other plant pathogens, the management of plant viral diseases based on direct methods have not been developed yet, so that viral diseases can be controlled by indirect strategies such as insect viral vector control or removing diseased plants (Aboul-Ata *et al*., 2011; Wang *et al*., 2011). The methods for detection and identification of viruses are critical in virus disease management (Aboul-Ata *et al*., 2011). Therefore, detection methods should be more convenient, effective, specific and permitted the use for detecting plant pathogens (McCartney *et al*., 2003).

A lot of methods have been developed to detect plant viruses, such as microscopical observation, serological techniques, molecular methods and so on (Lopez *et al*., 2008; Makkouk and Kumari, 2006; Webster *et al*., 2004). Among them, a number of methods for the diagnosis of plant viral diseases are reviewed in the following two sections, serological method and molecular method.

Serological methods:

Serological detection systems use specific antibody developed in animals in respond to antigens (Torrance, 1998). Viruses can be detected if viral antigens are used to develope antibody. In fact, these kinds of techniques have been used for the routine diagnostic tool. Many serological methods have been reported including enzyme-linked immunosorvent assay (ELISA), tissue blot immunoassay (TBIA) and quartz crystal microbalance immunosensors (QCMI).

ELISA. Common ELISAs are performed in polystyrene plate capable of binding antibodies or proteins with association of the enzyme-substrate reaction (Corning Life Science, 2001; Luminex, 2010). In order to get an accurate and reproducible result, the enzyme-substrate reaction needs to be optimized timing and development conditions (Corning Life Science, 2001). ELISA has been used as very popular assay to detect plant viruses within plant material, insect vectors, and seeds (Clark and Adams, 1977; Naidu and Hughes, 2001; Webster *et al*., 2004). Level of infection is measured based on the optical density (the degree of coloration) of ELISA reaction (Corning Life Science, 2001; Webster *et al*., 2004). Advantages of ELISA are that it is sensitive, a great number of samples can be examined at the same time (Vemulapati *et al*., 2014) little amount of antibody for the detection of diseases, and the process can be semi-automated (Naidu and Hughes, 2001). Specific antiserum has been developed against the target virus (Torrance, 1998). It has been employed for the detection of a lot of viruses including CMV, *Citrus tristeza virus* (CTV), *Potato leaf roll virus* (PLRV), *Potato virus X* (PVX), and *Potato virus Y* (PVY) (El-Araby *et al*., 2009; Sun *et al*., 2001). Large amount of sample for ELISA is needed for capturing antigen of interest from the sample compared to sample requiring for molecular methods and it takes about 2 days for diagnosis (Lievens *et al*., 2005; Luminex, 2010). Since ELISA is antiboy-antigen based assay, availability of antibody properly responding against the target agent is regarded as very important factor. ELISA often offers misdiagnosis due to false positive which is mainly resulted from non-specific reactions or cross-reactivity with certain factors in samples (Kfir and Genthe, 1993). Antibody used in ELISA can respond to many strains with an obvious different symptom because of lack of specificity. Therefore, strains of virus very related cannot be differentiated correctly by ELISA (Boonham *et al*., 2014). Although ELISA sensitivity was increased by adding some additives in extraction buffer (Fegla and Kawanna, 2013), ELISA is generally less sensitive when compared to molecular methods. Because of these reasons, although ELISAs have been widely used for diagnostic purpose up to date, the use of ELISA in terms of diagnosis seems to be gradually decreased. It is thought that alternative tools to be employed in coming age will be introduced in to a diagnostic market or more researches will be continued to overcome ELISA's shortcomings.

Tissue blot immunoassay (TBIA). Since principle of TBIA is the same with that of ELISA to which antibody is applied, TBIA has the same reliability to ELISA to detect plant viruses (Hančević *et al*., 2012). Major difference is that polystyrene plate is used as platform of ELISAs, whereas TBIA is performed on nitrocellulose and nylon membranes. That is reason that this assay is called as TBIA or TIBA (Webster *et al*., 2004). Like ELISA, TBIA also has necessary of a specific antibody to get rid of false positive and also needs large amount of virus concentration to reduce false negative. However, since TBIA has great benefits over ELISA in terms of detection time, cost, sensitivity and convenience, it has been applied for diagnosis of a number of viral diseases caused by *Bamboo mosaic virus* (BoMV), *Bean yellow mosaic virus* (BYMV), CTV, *Cymbidium mosaic virus* (CyMV), *Papaya ringspot virus* (PRSV), *Sweet potato feathery mottle virus* (SPFMV), and *Tomato spotted wilt virus* (TSWV) (Bove *et al*., 1988; Eid *et al*., 2008; Hančević *et al*., 2012; Lin *et al*., 1990; Makkouk and Kumari, 2006; Shang *et al*., 2011; Webster *et al*., 2004).

Quartz crystal microbalance immunosensors (QCMI). The QCM measures mass based on vibrations and frequency change in real time and it has been widely used to measure small mass in vacuum, gas, and liquid condition (Kurosawa *et al*., 2006; Mecea, 2005, 2006). Immunological combination with QCM results in QCMI as a mass-sensitive transducer device (Owen *et al*., 2007). Antigen-antibody binding reaction causes decreased quartz crystal oscillation frequency in positive reaction. QCMI, which offers some advantages, including high

sensitivity, real time output, portability, label-free entities, and low cost of operation, fabrication, and maintenance becomes attractive alternatives to conventional analysis methods (Chen and Tang, 2007; Lee and Chang, 2005; Tang *et al*., 2006). If the analytical signal is too weak to detect target materials, the detection sensitivity can be increased by introducing the signal-enhancing step (Kurosawa *et al*., 2006). QCMI shows high detection sensitivity for biological materials even viruses (Bachelder *et al*., 2005; Eun *et al*., 2002; Kleo *et al*., 2011; Lee and Chang, 2005; Owen *et al*., 2007; Su *et al*., 2003; Susmel *et al*., 2000; Uttenthaler *et al*., 2001). Because a detection instrument for QCM is potable and QCM coated with virus-specific antibodies to detect plant viruses has long life span, it can be used for on site detection of plant viruses (Becker and Cooper, 2011; Eun *et al*., 2002).

Since QCM has proven to be successful in detection of plant viruses, including *Cymbidium mosaic virus* (CyMV), TMV and *Turnip yellow mosaic virus* (TYMV), were detected using QCM (Dickert *et al*., 2004; Eun *et al*., 2002; Zan *et al*., 2012).

Molecular methods:

Molecular methods can be applied for diagnosis of many viral diseases when genetic information of viruses is available. As an alternative method to serological one, it is most commonly used in the laboratory due to high accuracy and sensitivity.

Polymerase chain reaction (PCR).

PCR and reverse transcription PCR (RT-PCR). PCR is a scientific technique used to amplify, or create millions of identical copies of a particular DNA sequence within a tiny reaction tube. Prior to the initiation of each new round for DNA amplification, the DNA is denatured, two sets of oligonucleotides (called primers) anneal to the denatured complementary strand. Then, primers lead DNA synthesis by the DNA polymerase. All reactions occur sequentially in template dependent manner. Thorough this, the target sequences of interesting DNA are exponentially amplified (Saiki *et al*., 1985, 1988).

PCR has been used as one of core techniques to molecular biology based-researches in a many of applications such as cloning, gene manipulation, gene expression analysis, genotyping, sequencing, and mutagenesis. In addition, PCR has also been used as a diagnostic tool to detect diseases (Makkouk and Kumari, 2006; Schaad and Frederick, 2002).

Currently, PCR is popular techniques for detection of plant viruses in the laboratory and is very commonly used in molecular experiments (Webster *et al*., 2004). PCR is currently the basis of all diagnostic methods, used with other detection methods (Lopez *et al*., 2008). The effective diagnostic methods of viruses, PCR is able to process by the specificity of the primers. PCR is proceeded through three steps, denaturation above 94°C, annealing of primers at 50–75°C (depend on primers) and elongation at 72°C (Makkouk and Kumari, 2006; McCartney *et al*., 2003).

RT-PCR used for the detection of RNA viruses requires reverse transcriptase which is added at the step of reverse transcription before the regular PCR step (Lopez *et al*., 2008; Webster *et al*., 2004). Since RT-PCR technique is sensitive, specific, and inexpensive compared to serological methods and is also more reliable than serological methods (Lievens *et al*., 2005; Lopez *et al*., 2008; McCartney *et al*., 2003), it has been developed and employed to detect many potato viruses such as PVX, PLRV, and PVS in stem or seeds of potato (Drygin *et al*., 2012; Ham, 2003; Peiman and Xie, 2006; Peter *et al*., 2009). Potato viruses within aphids, their vectors, can be detected by RT-PCR (Peter *et al*., 2009; Singh *et al*., 2004). In addition, RT-PCR to detect plant RNA viruses was used for quarantine purpose (Lee *et al*., 2011). This technique is able to detect five viruses not reported in Korea including *Cucumber vein yellowing virus* (CVYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Potato aucuba mosaic virus* (PAMV), *Potato yellow dwarf virus* (PYDV), and *Tomato chlorosis virus* (ToCV) (Lee *et al*., 2011).

Multiplex PCR. Two or more targets DNA or RNA can be detected at the same time via multiplex PCR in a single reaction (Lopez *et al*., 2008; Webster *et al*., 2004). This methods required several specific primers to detect over two viruses or bacteria (Li *et al*., 2011; Menzel *et al*., 2002; Qu *et al*., 2011; Singh *et al*., 2000). There are several examples of simultaneous detection of viruses and also other plant pathogens in one host (Singh *et al*., 2000).

The many major characterized viruses were simultaneously detected at diseased apple trees through multiplex-PCR (Menzel *et al*., 2002). In comparison multiplex-PCR with ELISA to detect plant viruses, *Apple chlorotic leafspot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), and *Plum pox virus* (PPV), infection rate reported from multiplex-PCR was approximately 16.7% while ELISA was 10% although the same samples have been used, indicating that multiplex-PCR was superior to ELISA in time of detection and in sensitivity (Yardimic and Culal-Klllc, 2011). Mumford *et al*. (2000) employed fluorescence to detect multiple viruses in real time. Despite this benefit, conventional PCR is used more than multiplex PCR, probably due to the technical difficulties of reaction mixture involving many compatible primers (Lopez *et al*., 2008). Moreover, it is difficult to design specific primer for each target DNA and to distinguish with the difference DNA amplification of each size of the gene (Lopez *et al*., 2008; Webster *et al*., 2004).

Nested PCR. The method is useful when the virus titre is very low, target gene is unstable, and can not be checked by electrophoresis due to low amplification product (Webster *et al*., 2004). The product from primary PCR amplification is used for second PCR amplification. However, the second reaction can be caused to face the risk of contamination (Lopez *et al*., 2008). Problems mentioned above can be solved by Nested PCR (Olmos *et al*., 1999). Several viruses, including PNRSV, PDV, PPV and CTV, were detected by this technique (Adkar-Purushothama *et al*., 2011; Helguera *et al*., 2001, 2002; Olmos *et al*., 1999). This nested PCR was combine with Immunocapture-RT PCR to increase sensitivity and to simplify preparation of sample

(Helguera *et al*., 2001, 2002). This method was applied to detect *Lettuce mosaic virus* (LMV) even in single aphids (Moreno *et al*., 2007).

Co-operational PCR (Co-PCR). Both co-operational PCR and nested-PCR require a tetra primer set (Olmos *et al*., 1999; Olmos *et al*., 2002). However, co-operational PCR needs one external and three internal primers instead of two external and two internal primers associated with nested-PCR (Olmos *et al*., 2002; Pantaleo *et al*., 2001). Since co-operational PCR uses four primers like nested-PCR, this technique has some benefits over conventional PCR (Lopez *et al*., 2008; Olmos *et al*., 2002). Benefits include a single reaction, minimization of contamination risks, high sensitivity similar to nested PCR, detection in real-time, and capability of coupling with dot blot hybridization (Bertolini *et al*., 2007; Lopez *et al*., 2008; Martos *et al*., 2011). In addition, Co-operational PCR can avoid false positive shown at nested-PCR (Olmos *et al*., 2002) and also can be applied to capillary air thermal cyclers which could not be applied for nest-PCR used to detect *Squash vein yellowing virus* (SqVYV) (Adkins *et al*., 2008).

Consequently, co-operational PCR needs less time than nested-PCR (Olmos *et al*., 2002). The major obstacle to use conventional PCR is existence of PCR inhibitors. This problem can be overcome by co-PCR with diluted samples. Undiluted samples showed a weak product by co-PCR whereas diluted samples gave better signal (Capote *et al*., 2009; Caruso *et al*., 2003). According to the detection of *Cherry leafroll virus* (CLRV), the sensitivity of co-PCR observed in virus detection is at least 100 times higher than RT-PCR and is similar with that of nested RT-PCR (Olmos *et al*., 2002). However, it is worthy to note that not all co-PCR showed higher sensitivity compared to RT-PCR (Capote *et al*., 2009).

Real-time PCR. Real-time PCR was developed as one of the technical methods to monitor the amplification products of PCR in real-time and also allows accurate quantification of PCR products (McCartney *et al*., 2003; Ruiz-Ruiz, 2009). Real-time PCR can be dramatically reduced detection time and can be used for small concentration of target gene making possible to diagnose (Heid *et al*., 2011; Lopez *et al*., 2008) because of no need the gel elcetrophoresis for the confirmation. It also has been known that it is faster than conventional PCR with less risk of contamination (Lopez *et al*., 2008). Although the real time monitoring curve was raised up as the DNA exponentially amplified, there are some drawbacks to use real-time PCR. One is that the amplification arrests when it reaches certain level, plateau (Gibson *et al*., 1996). Other disadvantage is that real-time method requires highly expensive equipment. Though these demerits, real-time PCR has been increasingly used because this method has been showed valuable detection for plant viruses (McCartney *et al*., 2003). *Citrus tristeza virus* (CTV) in different plant tissues and TMV in soil were detected by real-time PCR and quantified *Citrus leaf blotch virus* (CLBV) (Ruiz-Ruiz *et al*., 2007, 2009; Yang *et al*., 2012). It also used to discriminate two potato pathogenic bacteria on infected potato tubers (Qu *et al*., 2011).

Isothermal amplification.

Generally speaking, the use of PCR variants is increasing for the disease diagnosis. In order to complete the first round of PCR, PCR is necessary of 3 different temperatures for denaturalization of double stranded DNA, primer annealing to target DNA, and extension of DNA synthesis. Thus it needs expensive instruments that can control temperature precisely. Polymerase, which can amplify DNA, at constant temperature was discovered. It called Isothermal PCR. Many isothermal PCRs exist but here nucleic acids sequence-based amplification (NASBA) and loop-mediated isothermal amplification (LAMP) are described.

Nucleic acids sequence-based amplification (NASBA). NASBA, a primer-dependent continuous amplification, has been used for the direct amplification of RNA by PCR using a reverse transcriptase, RNase H and, T7 RNA polymerase (Compton, 1991). One of differences compared to conventional PCR is that it works at isothermal condition instead of thermal cycling. The other is that products by NASBA are antisense to the target viral sequences. Lopez *et al*. (2008) carried out the entire process at 41°C for 60 min and visualized a real-time assay using molecular beacons.

Since it is more sensitive than conventional PCR, the reaction time can be reduced (Vaskova *et al*., 2004). Real-time NASBA has been applied to detect plant viruses including *Strawberry vein banding virus* (SVBV), *Apple stem pitting virus* (ASPV) and PPV (Klerks *et al*., 2001; Leone *et al*., 1997; Olmos *et al*., 2007; Vaskova *et al*., 2004).

Loop-mediated isothermal amplification (LAMP). LAMP is performed at a constant temperature for one hour using the four primers (Notomi *et al*., 2000). The first product is formed in the loop formation and DNA has incessantly amplified from the first products resulted in various sized DNA structures (Fig. 1). The diagnosis, therefore, is possible although there is very small amounts of target gene (Parida *et al*., 2008; Tomita *et al*., 2008). The LAMP products from reaction can be detected by electrophoresis and observed a smear of multiple bands in a lane of positive LAMP reaction (Notomi *et al*., 2000; Parida *et al*., 2008; Tomita *et al*., 2008).

The LAMP assay has been recently applied for the rapid detection of several viruses in animal, such as *Canine parvovirus* (Cho *et al*., 2006). In addition, it has been used to determine sex of asparagus, genetically modified organisms (GMOs), and Phytoplasmas (Lee *et al*., 2009; Shiobara *et al*., 2011; Tomlinson, 2010). The RT-LAMP has been developed for simple monitoring of RNA viruses including PVY and PLRV (Ju, 2011; Nie, 2005).

Microarray (Oligonucleotide array).

Microarray is the evolved platform of the southern blotting technology. This technique used glass instead of nitrocellulose and nylon membrane as a supporter (Maskos and Southern,

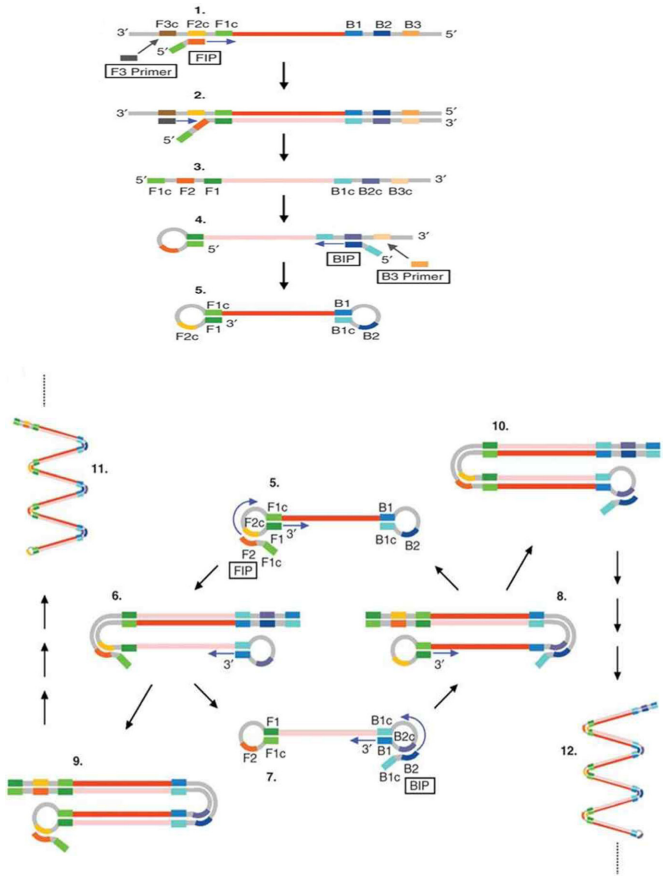

Fig. 1. Principle of LAMP-PCR. While DNA denaturation step (the double stranded DNA into a single strand) is vital for conventional PCR, LAMP-PCR does not require that. There are 11 steps for LAMP-PCR. 1, After FIP (one of the LAMP primers) anneals to the complimentary sequence of target DNA conditioned around 65°C, DNA strand is synthesized from the 3′ end of the F2 in the FIP by DNA polymerase with strand displacement activity; 2, The F3 anneals to the F3c region on the target DNA and begins releasement of FIP-linked complementary strand synthesized at step 1; 3–4, The released single strand forms a loop structure at the 5′ end because the F1c is complementary to F1 and after BIP anneals to its complimentary, new DNA strand is synthesized from the 3′ end of the B2 in the BIP by DNA polymerase; 5, The B3 anneals to the B3c region, outside of BIP, on the target DNA and begins synthesis of DNA strand (BIP-linked complementary strand), forming dumbell-like structure with stem-loop at each end due to complimentary of F1 and B1 to F1c and B1c, respectively. This structure serves as the starting material for the amplification; 6-12, FIP anneals to the stem-loop DNA and leads strand displacement DNA synthesis and, releasing the previously synthesized strand which forms a stem-loop structure at the 3′ end owing to B1c is complementary to B1. Later complementary strand with FIP is released. The released strand forms dumbbell-like structure with stem-loop at each end since F1 and B1 are complimentary to F1c and B1c, respectively. Because DNA synthesis continues, there are various sized structures (Under permission form Eiken Chemical Co, Ltd. 2005; Eiken, 2005).

1992) and was initially developed for differentiation of messenger RNA expression (Schena *et al*., 1995). Later, this technique demonstrated the potential to detect viral pathogens without amplification of viral RNA (Nam *et al*., 2014). Oligochips used in oligonucleotide array is composed of thousands of specific probes spotted onto a solid surface like a glass plate (Lopez *et al*., 2008). Synthesized single stranded DNA probes with about 25bp to 70bp nucleotides are hybridized with the virus extracted from plant (Lee *et al*., 2003; Wang *et al*., 2002, 2003). Most drawback is cost since it requires the highly sophisticated processing machine for spotting probes and reading reactions and also needs dust-free room (Wang *et al*., 2002). The other problem is construction of oligonucleotide to be hybridized to target DNAs in terms of specificity and sensitivity (Dugat-Bony *et al*., 2012). Because of those, it has not been widely used and is still under research phase. However, some trials to use this method can be found because it is able to detect both known and unknown sequences in environmental samples, resulted in identifying unknown viruses by Oligo-chip (Boonham *et al*., 2007; Dugat-Bony *et al*., 2012; Nam *et al*., 2014; Schena *et al*., 1995). This method was used detection for potato viruses such as *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus S* (PVS), PVX, PVY and PLRV and cucurbit-infecting plant viruses (Bystricka *et al*., 2005; Lee *et al*., 2003). It is also able to identify any plant virus at the genus level and can differentiate relevant strains (Hammond, 2011; Wang *et al*., 2002; 2003; Zhang *et al*., 2010). Recently, a large-scale oligonucleotide (LSON) chip were developed to detect and to identify 538 plant viruses (Nam *et al*., 2014). Since this method has the great potential to detect all the pathogen in animals and human beings, and plants in a single chip, it can be extended for quarantine purposes.

Conclusions

Economic loss has been estimated more than several billion dollars per year worldwide due to plant viral diseases and there is no commercialized chemical to manage those. Plant diseases caused by viruses can be effectively controled when means of manage are applied at the initial step of viral disease development or by planting virus-free crops. This is reason why accurate diagnosis is important. Symptomatic diagnosis is still useful but often has erroneous results, because of confusion associated with high variable symptoms by interactions between host and virus or by abiotic stresses. Therefore, reliable diagnostic platforms which can be accepted officially are required.

The methods based on serological principle and molecular biology have been used for virus diagnosis. ELISA associated with serology was initiated and adapted as a diagnostic tool worldwide since it is easy to use with durability. After PCR invented, PCR based diagnostics have been adapted as a diagnostic system comparable with ELISA even becoming predominant method. There are several reasons for this change. PCR is standardized in industrial level as ELISA resulting in worldwide use in diagnostic facilities. Futhermore, PCR-based assay has better sensitivity over ELISA and even faster. Isothermal nucleic acid amplification methods including LAMP are under development for virus detection because it is faster and has greater sensitivity

over conventional PCR. Although microarray invented to examine variations in level of messenger RNA expression is still under research phase to increase sensitivity for differentiating target signal with background due to host, which is one of drawback for microarray compared to PCR method, it has been showed highly potential to use as a diagnostic tool.

Many kinds of techniques are available now and are developing for the diagnostic purpose. Since there is no an ideal detection method to fulfil all requirement to detect, it is very important to develop appropriate and effective techniques which can be applied for management of viral diseases in worldwide level. By doing that sustainable agriculture will be achieved.

Acknowledgement

This work was supported by a Grant from the Next-Generation Biogreen 21 Program (PJ008063), Rural Development Administration, Republic of Korea.

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