A Review of Detection Methods for the Plant Viruses

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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 response to antigens (Torrance, 1998). Viruses can be detected if viral antigens are used to develop 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 (TBA) 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 antibody-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 (TBA). Since principle of TBA is the same with that of ELISA to which antibody is applied, TBA 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 TBA is performed on nitrocellulose and nylon membranes. That is reason that this assay is called as TBA or TIBA (Webster et al., 2004). Like ELISA, TBA 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 TBA 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