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Biosensors for plant pathogen detection

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ABSTRACT

Infectious plant diseases are caused by pathogenic microorganisms such as fungi, bacteria, viruses, viroids, phytoplasma and nematodes. Worldwide, plant pathogen infections are among main factors limiting crop productivity and increasing economic losses. Plant pathogen detection is important as first step to manage a plant disease in greenhouses, field conditions and at the country boarders. Current immunological techniques used to detect pathogens in plant include enzyme-linked immunosorbent assays (ELISA) and direct tissue blot immunoassays (DTBIA). DNA-based techniques such as polymerase chain reaction (PCR), real time PCR (RT-PCR) and dot blot hybridization have also been proposed for pathogen identification and detection. However these methodologies are time-consuming and require complex instruments, being not suitable for in-situ analysis. Consequently, there is strong interest for developing new biosensing systems for early detection of plant diseases with high sensitivity and specificity at the point-of-care. In this context, we revise here the recent advancement in the development of advantageous biosensing systems for plant pathogen detection based on both antibody and DNA receptors. The use of different nanomaterials such as nanochannels and metallic nanoparticles for the development of innovative and sensitive biosensing systems for the detection of pathogens (i.e. bacteria and viruses) at the point-of-care is also shown. Plastic and paper-based platforms have been used for this purpose, offering cheap and easy-to-use really integrated sensing systems for rapid on-site detection. Beside devices developed at research and development level a brief revision of commercially available kits is also included in this review.

1. Introduction

Plant pathogens are one of the causes for low agricultural productivity worldwide. Main reasons are new, old and emerging plant infectious diseases. Their rates of spread, incidence and severity have become a significant threat to the sustainability of world food supply (Pimentel et al., 2005; Oerke, 2006; Roberts et al., 2006; Savary et al., 2012). Despite the lack of sufficient information for the economic losses, it was reported from plant disease loss estimates in U.S state of Georgia that total losses caused by plant diseases and their control costs reached roughly 647.2 million dollars in 2006 and then continued up to 821.85 million dollars in 2013 (Martinez, 2006, 2013). Top ten list of economically and scientifically important plant pathogens includes fungi, bacteria and viruses (Dean et al., 2012; Mansfield et al., 2012; Scholthof et al., 2011; Rybicki, 2015) (Table 1).

Plants display different symptoms on leaves, stems and fruits due to plant disease infections (López et al., 2003; Al-Hiary et al., 2011) (Fig. 1). These symptoms are particularly useful for visual observation as a conventional first step for plant disease diagnosis but it fails in detecting the presence of pathogen in early infection stages when plant infections are symptomless..

Early detection of plant pathogens plays an important role in plant health monitoring. It allows to manage disease infections in greenhouse systems and in the field during different stages of plant disease development and also to minimize the risk of the spread of disease infections as well as to prevent introduction of new plant diseases, especially quarantine pathogens at country boarder (Anderson et al., 2004; Strange and Scott, 2005; Brassier, 2008; Vincelli and Tisserat, 2008; Miller et al., 2009). Many strategies have been widely used for diagnosing plant disease problems including DNA-based methods and immunoassays, for the detection of pathogen protein and nucleic acid extracted from infected plant materials, as direct laboratory based techniques in addition to visual inspection of plant symptoms in the field (López et al., 2003) (Fig. 2A).

On the other hand there are other indirect strategies based on analysis of volatile organic compounds (VOC) that plants release as

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Table 1
Top ten important plant pathogenic bacteria, fungi and viruses published by Molecular Plant pathology (Dean et al., 2012; Mansfield et al., 2012; Scholthof et al., 2011; Rybicki, 2015).

Plant pathogen	Fungi	Bacteria	Virus
1	Magnaporthe oryzae	Pseudomonas syringae	Tobacco mosaic virus
2	Botrytis cinerea	Ralstonia solanacearum	Tomato spotted wilt
3	Puccinia spp.	Agrobacterium tumefaciens	Tomato yellow leaf curl
4	Fusarium graminearum	Xanthomonas oryzae	Cucumber mosaic
5	Fusarium oxysporum	Xanthomonas campestris	Potato virus Y
6	Blumeria graminis	Xanthomonas axonopodis	Cauliflower mosaic
7	Mycosphaerella graminicola	Erwinia amylovora	African cassava mosaic
8	Colletotrichum spp	Xylella fastidiosa	Plum pox
9	Ustilago maydis	Dickeya (dadantii and solani)	Brome mosaic
10	Melampsora lini	Pectobacterium carotovorum	Potato virus X

defense mechanism against pathogen attack (Scala et al., 2013) (Fig. 2B). Some recent reviews have described in detail the strategies for monitoring of volatile compounds in plants for disease detection (Sankaran et al., 2010; Nezhad, 2014; Fang and Ramasamy, 2015; Martinelli et al., 2015).

Several previous studies addressed plant disease diagnosis and pathogen detection using nucleic acid -based methods, mainly consisting of polymerase chain reaction (PCR) followed by DNA hybridization detection, to determine the genetic content of pathogen (Lin et al., 1990; Minsavage et al., 1994; Anwar Haq et al., 2003; Das, 2004; Teixeira et al., 2005; Li et al., 2006; Lacava et al., 2006; Saponari et al., 2008; Urasaki et al., 2008; Fang et al., 2009; Li et al., 2009; Ruiz-Ruiz et al., 2009; Gutiérrez-Aguirre et al., 2009; Yvon et al.,2009). Alternatively, immunoassays, also known as serological assays, including enzyme-linked immunosorbent assay (ELISA), lateral flow devices (LF), tissue print ELISA or direct dot blot immunoassay (DTBIA) have been used to detect the pathogen antigens (Avrameas, 1969; Van Weemen and Schuurs, 1971; Garnsey et al., 1993; Cambra et al., 2000; Nolasco et al., 2002; Holzloehner et al., 2013; Escoffier et al., 2016). Immunoassay technology using monoclonal antibodies offers a high specificity for plant virus detection, being ideal for testing large scale plant samples and for the on-site detection of plant pathogens, as done with tissue print ELISA and LF devices. In contrast, nucleic acid based methods are more accurate and specific enough to detect single target pathogen within a mixture containing more than one analyte and highly effective for detection of multiple targets.

In spite of these advantages, molecular detection methods have some limitations in detecting pathogens at low titres in materials such as seeds and insect vectors or at early infection stages. Furthermore, false negative results can be produced from cross contamination with PCR reagents which completely block amplification of target DNA, while false positive results can be generated by cross-amplification of PCR-generated fragments of non-target DNA. Another limitation is related to the disability to apply PCR for plant pathogen detection in the field (Louws et al., 1999; Schaad and Frederick, 2002; López et al., 2003; Martinelli et al., 2015). To overcome such limitations, innovative and portable biosensors have emerged in the last years, being widely used as diagnostic tools in clinical, environmental and food analysis.

Pathogen biosensing strategies are based on biological recognition using different receptors such as antibodies, DNA probe, phage and others (Eggibs, 2002; Sadanandom and Napier, 2010; Singh et al., 2013) (Fig. 3).

Antibody-based biosensors can allow sensitive and rapid qualitative and quantitative analysis of pathogens offering also label-free possibilities. It is important to note that this general approach is limited by the quality of the antibody employed and its storage condition that could affect antibody stability. Also pathogen size can interfere in some measurements such as the ones based on surface plasmon resonance (SPR). DNA based biosensors show advantages over antibody based ones mostly related to their better sensitivity thanks to the use of nucleic acid amplification techniques, which allows to detect plant pathogen before appearance of disease symptoms. However, they have some limitations related to the selection and synthesis of specific DNA probes as well as to the fact that detecting short DNA sequence of long double stranded DNA is a common problem in applying biosensing systems for DNA detection (Skottrup et al., 2008; Fang and Ramasamy, 2015; Hushiarian et al., 2015). Recently, phage-based DNA biosensor for sensing and targeting bacterial plant pathogens has been reported

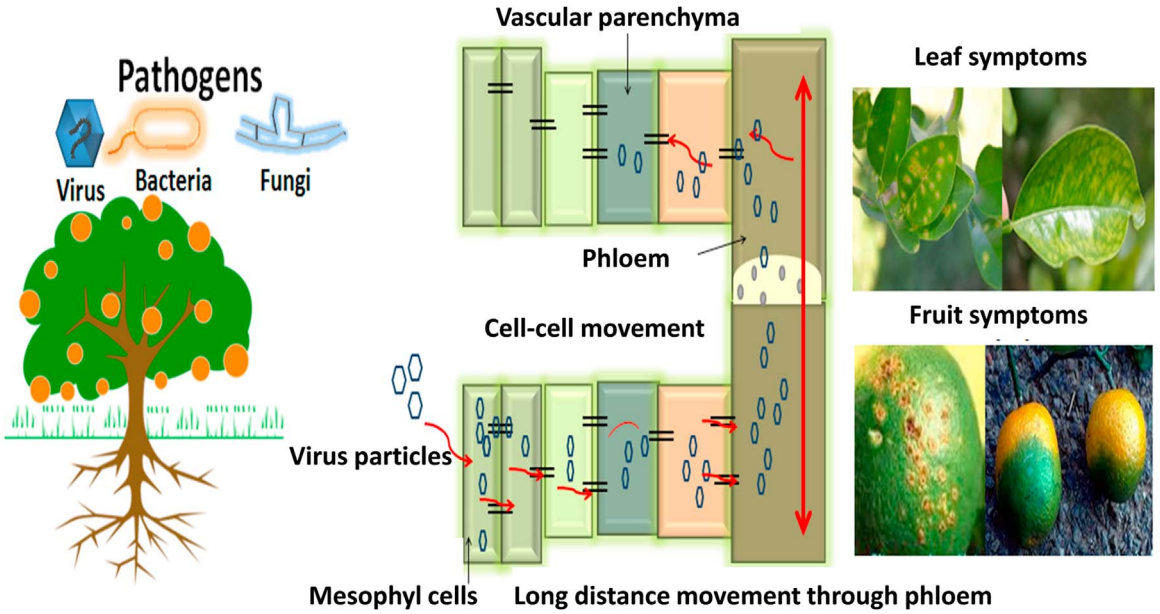

Fig. 1. Illustration of bacterial disease symptoms on citrus leaves and fruits. Adapted with permission from < http://www.crec.ifas.ufl.edu > (Viewed on Sunday, 22, May 2016).