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Fungal disease detection in plants: Traditional assays, novel diagnostic techniques and biosensors

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ABSTRACT

Fungal diseases in commercially important plants results in a significant reduction in both quality and yield, often leading to the loss of an entire plant. In order to minimize the losses, it is essential to detect and identify the pathogens at an early stage. Early detection and accurate identification of pathogens can control the spread of infection. The present article provides a comprehensive overview of conventional methods, current trends and advances in fungal pathogen detection with an emphasis on biosensors. Traditional techniques are the "gold standard" in fungal detection which relies on symptoms, culture-based, morphological observation and biochemical identifications. In recent times, with the advancement of biotechnology, molecular and immunological approaches have revolutionized fungal disease detection. But the drawback lies in the fact that these methods require specific and expensive equipments. Thus, there is an urgent need for rapid, reliable, sensitive, cost effective and easy to use diagnostic methods for fungal pathogen detection. Biosensors would become a promising and attractive alternative, but they still have to be subjected to some modifications, improvements and proper validation for on-field use.

1. Introduction

Fungi are the most diverse group of plant pathogens accounting for 70–80% of plant diseases (Persley, 1993). Over 20,000 species of fungi are parasitic in nature and can cause diseases in crops and plants (USEPA, 2005). Fungi can survive in a dormant state on living or dead plant tissues until conditions become favorable for their proliferation. They can penetrate into plant tissue or grow on the surface. The fungal disease cycle in plants is highlighted in Fig. 1. Fungal spores easily spread by wind, water, soil and animals, thereby reaching the neighbouring fields and damaging the entire harvest. While many fungi play useful roles in plant growth, especially by forming mycorrhizal associations with the host plant's roots, others cause common plant diseases such as anthracnose, rusts, leaf spots, wilts, curls, blight, scab, smuts, galls, cankers, root rots, damping off, dieback and mildews.

The fungal diseases can, at times, seriously compromise food security. For example, potato late blight, caused by *Phytophthora infestans* in the mid-1840s became historically significant in Europe. In Ireland, nearly a million people died of starvation and over a million attempted to migrate elsewhere (Fry and Goodwin, 1997). There have been other disasters accounts such as the Great Bengal Famine (1943) in India (Padmanabhan, 1973) and the southern corn leaf blight epidemic (1970–1971) in USA (Ullstrup, 1972). In India, about 2 million people died due to the high dependence of most of the population on a single crop, rice that was attacked by the fungus *Cochliobolus miyabeanus*. In USA, the maize crop was completely destroyed by another fungus from the same genus, *Cochliobolus heterostrophus*, thereby severely affecting on the agricultural economy. The above examples demonstrate that in parts of the world where a large proportion of the population is dependent on a single crop or a few crops then they are at risk, since a crop failure due to devastating disease can lead them to starvation.

A number of studies have demonstrated the devastating effect these fungi have on crop yield. Rice, a staple food of the world's half population is attacked by *Pyricularia oryzae* causing rice blast and leading to a loss of 10–30% of the crop every year (Talbot, 2003). In Bhutan (1995) more than 700 ha of rice of different genotypes was affected, resulting in losses of 1090 t (Thinlay et al., 2000). Foot rot disease caused by *Phytophora palmivora* in the year 1956 destroyed about 50% of the pepper gardens in Kuching, Malaysia resulting in a loss of 7000 t of pepper (Purseglove et al., 1981).

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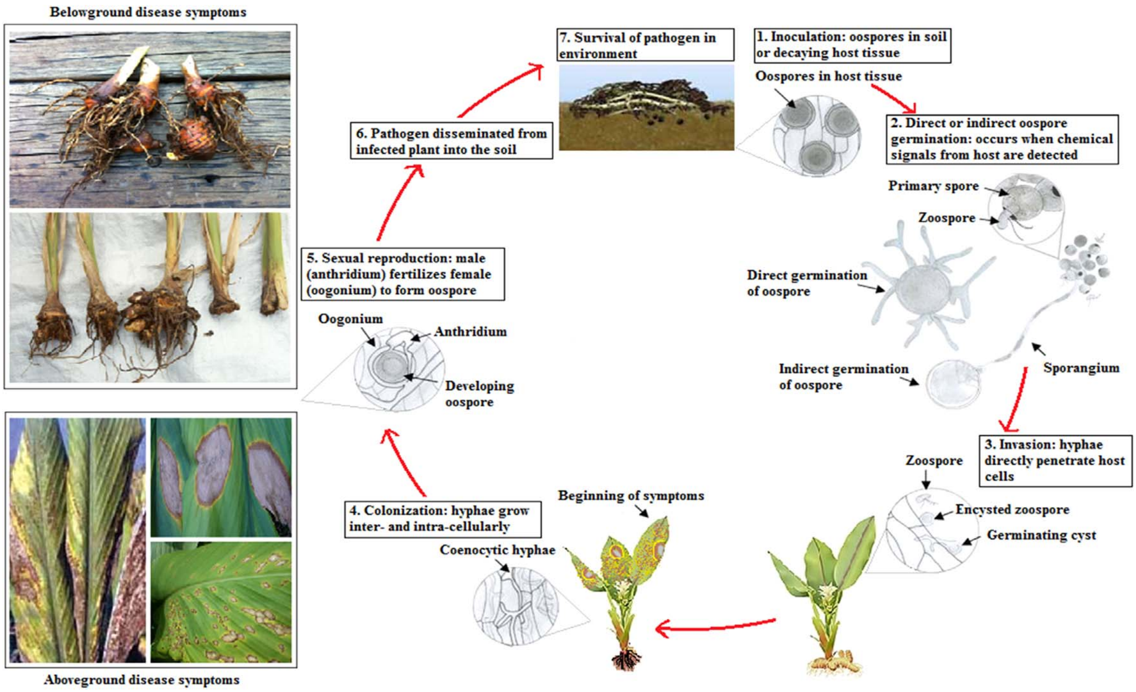

Fig. 1. Fungal disease cycle in a plant.

Moreover, once a fungus is introduced into the plant it persists for a long time. Disease control becomes a challenging task due to fungicide resistance. Chestnut blight was introduced accidentally into New York City in the year 1904, marking the beginning of an ecological disaster in the forests of eastern North America (Milgroom and Cortesi, 2004). This devastating epidemic was caused by *Cryphonectria parasitica* on chestnut trees that was introduced into America from China. The Chinese trees were resistant to the blight, but American chestnut trees were not. In less than 40 years, 30 million acres of chestnut trees had died. Chestnut blight still remains a problem in the eastern United States. Similarly, *Plasmopara viticola*, a fungus native to North America was accidentally introduced into Europe at the end of the 19th century, where it caused powdery mildew and downy mildew and devastated the wine industry until the Bordeaux mixture was found to control them (Gessler et al., 2011).

The recent attention towards food security issues provides an intense motivation in the area of plant fungal pathogen detection. Therefore, a comprehensive literature study has been carried out aiming to present an overview of the plant fungal pathogen detection over the past 15 years (Fig. 2). Pathogen detection using conventional techniques are time-consuming, expensive and often require well trained trainers for the sample pre-treatment steps. Biosensors offer advantages over current analytical methods by offering intrinsic features like sensitivity, rapid detection ability, cost effective and portability.

In this review, we have discussed the major fungal diseases in plants, outbreaks caused by fungal pathogens in plants and various fungal detection techniques. This study describes two approaches to detect plant fungal diseases. The first approach involves conventional methods, application of spectroscopic and imaging techniques and application of volatile organic metabolites as possible biomarkers for disease detection, including their strengths and weaknesses. The second approach describes the current progress in the biosensors in the field of fungal pathogen detection. This paper reviews majorly on recent technological advances in detection, identification and quantification of fungal pathogens, with an emphasis on biosensors.

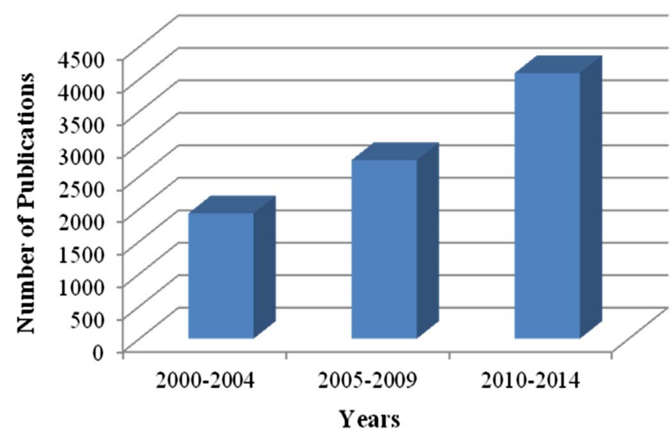

Fig. 2. Number of published papers during the period 2000–2014. These data were obtained by searching papers using keywords “plant fungal pathogen detection” on Scopus database, Elsevier.

2. Diagnosis of fungal diseases in plants

The ability to detect, identify and quantify plant pathogens accurately is the milestone of plant pathology. The reliable identification of the organisms responsible for a plant disease is an essential prerequisite to the implementation of disease management strategies. Therefore, in the diagnosis of plant fungal infections, it is essential to carry out a correct disease detection process. Because many fungal pathogens produce similar symptoms and therefore it is important to be able to distinguish between different species.

2.1. Conventional methods towards plant fungal pathogen detection

Conventional methods for detection and identification of fungal pathogen causing diseases in plants, mainly rely on morphological, microbiological and biochemical identifications. Conventional methods being used for the detection of fungal pathogens are represented in Fig. 3, which includes identification of fungal pathogens by interpreting the visual symptoms, culturing and plating methods and isozyme