



Advance Method of Plant Disease Detection

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Plant diseases cause major production and economic losses in agriculture and forestry. For example, soybean rust (a fungal disease in soybeans) has caused a significant economic loss and just by removing 20% of the infection, the farmers may benefit with an approximately 11 million-dollar profit (Roberts et al., 2006). It is estimated that the crop losses due to plant pathogens in United States result in about 33 billion dollars every year. Of this, about 65% (21 billion dollars) could be attributed to non-native plant pathogens (Pimentel et al., 2005). Some of the diseases caused by introduced pathogenic species are chestnut blight fungus, Dutch elm disease, and huanglongbing citrus disease (Pimentel et al., 2005, Li et al., 2006).

The bacterial, fungal, and viral infections, along with infestations by insects result in plant diseases and damage. There are about 50,000 parasitic and non-parasitic plant diseases of plants in United States (Pimentel et al., 2005). Upon infection, a plant develops symptoms that appear on different parts of the plants causing a significant agronomic impact (López et al., 2003). Many such microbial diseases with time spread over a larger area in groves and plantations through accidental introduction of vectors or through infected plant materials.

After the onset of plant disease symptoms, the presence of disease in plants is verified using disease detection techniques. Presently, the plant disease detection techniques available are enzyme-linked immunosorbent assay (ELISA), based on proteins produced by the pathogen, and polymerase chain reaction (PCR), based on specific deoxyribose nucleic acid (DNA) sequences of the pathogen (Prithiviraj et al., 2004, Das, 2004, Li et al., 2006, Saponari et al., 2008, Ruiz-Ruiz et al., 2009, Yvon et al., 2009). In spite of availability of these techniques, there is a demand for a fast, sensitive, and selective method for the rapid detection of plant diseases. Disease detection techniques can be broadly classified into direct and indirect methods. summarizes some of these methods of disease detection. An advanced plant disease detection technique can provide rapid, accurate, and reliable detection of plant diseases in early stages for economic, production, and agricultural benefits.

In the present paper, advanced techniques of ground-based disease detection that could be possibly integrated with an automated agricultural vehicle are reviewed. In ground-based disease detection studies, both field-based and laboratory-based experiments are discussed in this paper. The field-based studies refer to studies that involve spectral data collection under field conditions, whereas laboratory-based studies refer to data collection under laboratory conditions. The laboratory-based experiments provide strong background knowledge (such as the experimental protocol and statistical algorithm for classification) for the field-based applications.

Traditional Plant Disease Detection Techniques

Primarily, the first step toward disease detection is by visually observing the symptoms present in the plants. While this can provide some direction, the potential difficulty lies in effectively addressing the challenge. Visual observation does not provide any specific information about the microorganism causing the disease as well as the period of infection. Visual observation of plant symptoms has limitations in providing specific information about the causative agent and the stage of infection. This method potentially leads to inaccuracies because it heavily relies on the expertise of the observer. Therefore, more advanced and standardized techniques for pathogen identification, detection, and quantification are necessary to overcome these limitations and ensure more precise and reliable disease diagnosis. Some of the previously used methods apart from visual observation of symptoms include microscopic evaluation of the morphological characteristics to identify pathogens, culturing on growth media, and serological, molecular, and phenotyping. While some pathogens can be detected using a growth medium when applicable, multiple approaches can be utilized to determine the specific disease.

However, like any other approach, traditional methods also have their limitations and challenges. Time consumption, reliance on bulky machinery, and the need for expert personnel, as well as the detection of targeted and non-targeted pathogens, are some of the major challenges associated with these methods.

Addressing these challenges is essential to fully harness modern detection techniques and their potential for disease detection and management.

Advanced Techniques

Accurate and rapid identification of pathogens is essential in applying the most appropriate disease management to produce quality crops. Conventional methods used over the years to detect different plant pathogens may include; visual observation, microscopy, mycological assays, plant indicator tests, and more. However, plant disease diagnosis based on phenotypic features is not always reliable and has some limitations in time and accuracy. While some common plant diseases can be easily identified in the field with a trained eye, many symptoms displayed by unhealthy plants could also be due to environmental stress, poor soil conditions, insects and pests, chemical damage from fertilizers or fungicides, and even more than one pathogen can attack a plant. Also, some phytopathogens can cause disease with asymptomatic or weakly characteristic symptoms at the beginning of development. Thus, the traditional forms of detection are at a disadvantage, as it becomes difficult to diagnose the diseases and identify their pathogens accurately. In the last two decades, with technological advancement came an improvement in rapid disease diagnosis techniques. Different phytopathogens, including fungi, bacteria, and viruses, can be identified using molecular and Immunological methods. These methods are highly effective for accurately identifying a pathogen at the species level. They provide real-time diagnosis, and the sensitivity of these analyses is much higher than that of conventional methods, which allows for the rapid and accurate detection of pathogens even in asymptomatic plants that may harbor relatively low pathogen populations.

Fluorescence *in Situ* Hybridization (FISH): Fluorescence *in situ* hybridization (FISH) assays using oligonucleotide probes targeting rRNA were first introduced in 1969. It examines the formation and detection of RNA-DNA or DNA-DNA nucleotide complementary hybrids in cells utilizing radioactively labeled oligonucleotides as probes.

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA is a serological technique introduced in the 1970s and has since become the most widely used laboratory method for screening viruses in plant samples. Although ELISA was developed to study viruses that have

characteristics that make early diagnosis challenging, this assay can also be used for detecting other plant pathogens, like bacteria and fungi

Polymerase Chain Reaction (PCR): Since the introduction of the polymerase chain reaction (PCR) technology for the development of monoclonal antibodies and amplification of nucleic acid sequences by Nobel laureate Kary Mullis in 1993, it has had a profound impact on plant disease diagnosis. PCR was initially used to detect highly specific diseases caused by bacteria and viruses because of its high accuracy in DNA hybridization and replication

Recombinase Polymerase Amplification (RPA): Molecular techniques such as PCR and qPCR are widely used and have been demonstrated to be highly specific and efficient tools for diagnostics. However, the limitations of these methods include the need for a costly thermal cycler, stringent thermal cycling conditions, high-quality nucleic acids as a starting point, and a skilled operator, and they are relatively time-consuming.

Remote Sensing (RS) and Imaging Technologies: Remote sensing means sensing things from a distance. The “American Society for Photogrammetry and Remote Sensing (ASPRS)” defined remote sensing as “the art, science, and technology of obtaining reliable information about physical objects and the environment, through the process of recording, measuring and interpreting imagery and digital representations of energy patterns derived from non-contact sensor systems”

Nanotechnology and Biosensors

The realm of nanotechnology has spearheaded the creation of innovative concepts and agricultural commodities, holding immense promise in addressing the aforementioned challenges. While nanotechnology has made significant strides in medicine and pharmacology, its application in agriculture has garnered relatively less attention. Nanotechnology encompasses the exploration and advancement of research and technology on the atomic, molecular, and macromolecular levels, enabling precise manipulation and examination of structures and devices within the range of 1 to 100 nanometers. Although this novel application is relatively new in disease management, it can be explored for various disease diagnoses, monitoring, and projecting ahead of disease breakout. The integration of nanotechnology in agriculture is currently under exploration for a spectrum of applications, encompassing the delivery of plant hormones, facilitation of seed germination, optimization of water management, transfer of target genes, utilization of nano barcoding, deployment of nanosensors, controlled release of agrichemicals. So, in plant disease management, Nanophytopathology is the use of nanotechnology to protect plants, detect diseases, and provide cures to the plants, thereby safeguarding the crops against widespread disease while ensuring effective crop protection

References

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