ISSN(Online): 2320-9801
ISSN (Print) : 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Website: www.ijircce.com

Vol. 7, Issue 3, March 2019

Methods for Plant Disease Detection: A Review

Sanjana G[1], Kala L[2]

P.G. Student, Department of Electronics and Communication Engineering, NSS College of Engineering, Palakkad, Kerala, India[1]

Associate Professor, Department of Electronics and Communication Engineering, NSS College of Engineering, Palakkad, Kerala, India [2]

ABSTRACT: Plant pathogens are major threat to the food production and supply. Pathogens such as fungus, bacteria and virus causes different kind of infections in plants leading to the loss in quantity and quality of production. The only key method is to identify the diseases effected in plants at an early stage and taking necessary actions for preventing the losses in production. The manual monitoring does not give successive results all the time so efficient methods are required for the plant disease detection. This paper reviews the different types of direct and indirect methods for plant disease detection. The direct method involves serological and molecular methods whereas the indirect method includes the imaging and spectroscopy techniques.

KEYWORDS: Plant pathogens; disease detection; serological methods; molecular methods; fluorescence in situ hybridization; immunofluorescence.

I. INTRODUCTION

Agriculture is a main source of income in India and also it is a major part of food supply. Around 70% of Indian economy depends on agriculture. Diseases in plant is a major issue in the production of food. This leads to severe loss in agriculture. So using new technologies is a remedy for this problem.

Plants are a vital source of energy which plays a crucial role in environmental issue like global warming. Plants are affected by different varieties of diseases which may be due to fungal, bacterial infections or due to the increase in population and changing climatic conditions [1]. The quantity and quality of production will be affected by this problem [2]. Most of the diseases occurs in the leaf part of plants rather than fruit, stem etc. Infections in leaves may be due sudden environmental changes such as rapid temperature variations, heavy rainfall or due to some insects or pests [4]. These diseases have to be detected in an early stage when the symptoms occur for its efficient eradication. There are several methods for detecting the diseases in plants. The first and foremost method is the optical observation which is not a fast and accurate method for all the diseases and also the field visit of experts are required for this which is a time consuming task. There are diseases which cannot be identified with the naked eyes and also, this type of disease detection is possible only after the major damage of the crops [3]. In the case of field visit even the expertise people can also fail to a successful diagnosis of plant diseases. So there comes the need of an automated method for disease detection and classification. An early stage detection of diseases in plants can help to provide early treatment and also can reduce the use of pesticides in the agricultural fields. Different techniques are available for the detection of plant diseases. Significant developments have been made in the field of agriculture by using the image processing technologies.

II. RELATED WORK

In paper [5], a brief description of some direct and indirect methods for the detection of plant diseases caused by the bacteria, virus and fungi is given. In paper [10], the author suggested the flow cytometry technique which has the

ISSN(Online): 2320-9801
ISSN (Print) : 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Website: www.ijircce.com

Vol. 7, Issue 3, March 2019

advantage of both the microscopy and biochemical analysis. They used the flow cytometry technique to detect and enumerate the viable bacteria in the filtered compost extracts. Paper [13] describes the Enzyme Linked Immunosorbent assay method for the detection and assay of plant viruses using enzyme labelled antibodies. This method enables a number of morphologically different viruses in purified preparations. In paper [14], a sensitive and rapid immunofluorescence method for the detection of uredinispores of fungi is given. Paper [16], describes various molecular methods. PCR allows the amplification of number of copies of specific DNA sequence by denaturation process, the PCR technic provides the detection and identification of pathogenic fungi. Paper [22] describes the imaging technique used for plant disease detection. These technique monitors the changes in water stature, accumulation of secondary metabolites etc. in order to get the early signs of stress. Paper [29] briefs the spectroscopy techniques. This paper gives the various applications and limitations of the fluorescence and Vis/IR spectroscopy. Both the techniques are non-invasive disease detecting tools where the Vis/IR technique is a least expensive technique and also it is easy to adapt than other techniques.

III. METHODS OF PLANT DISEASE DETECTION

The plant disease detection involves both direct and indirect method for the identification of diseases.

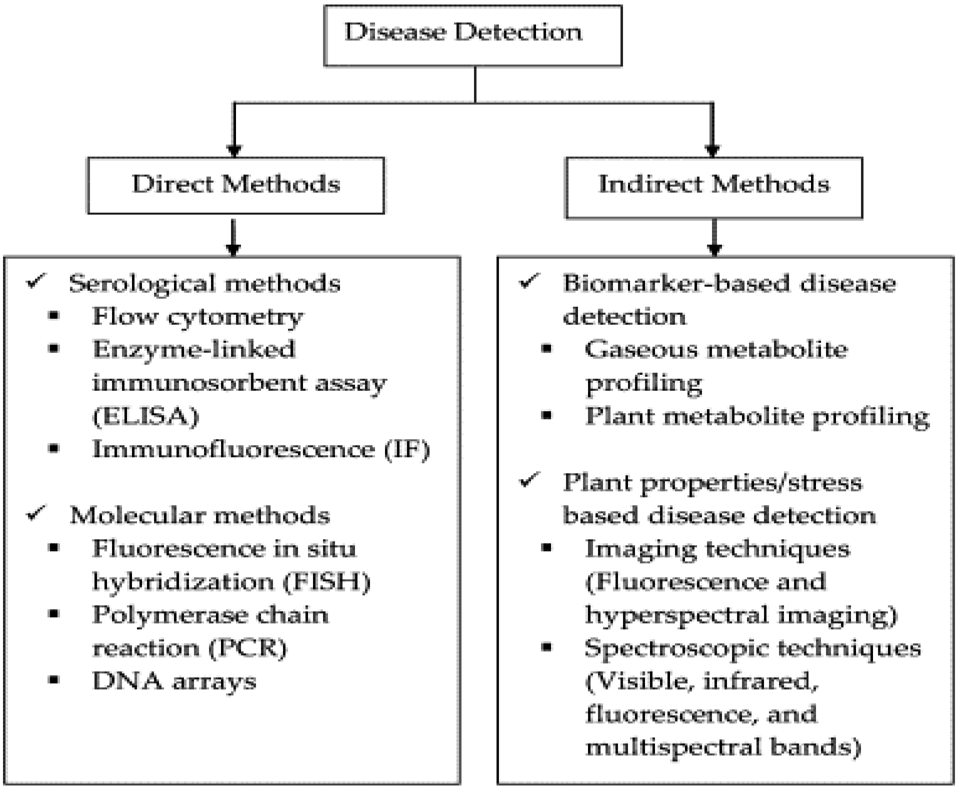

Fig.1: A review of advanced techniques for detecting plant diseases [8].

The direct method consists of the serological and molecular method. These two method can be used for the disease detection both in plants and animals [5]. The serological methods are commonly used for plant virus detection [6]. The second method is the Molecular method which is used to detect the plant pathogenic fungi [7]. Apart from the direct disease detection methods, the indirect methods use various parameters such as temperature variations, morphological changes, change in transpiration rate etc.

ISSN(Online): 2320-9801
ISSN (Print) : 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Website: www.ijircce.com

Vol. 7, Issue 3, March 2019

IV. DIRECT DETECTION METHODS

The direct method involves the molecular and serological techniques. The disease causing pathogens are detected directly and also these methods are used for getting high throughput analysis. [5]

A. *SEROLOGICAL METHODS*

The serological method which is used for the plant virus detection includes various types such as Flow cytometry, ELISA and Immunofluorescence assay. In the serological method, the reaction between the virus antigens and some antibodies is made used for the detection of diseases affected to plants [8].

- *Flow Cytometry*

  Flow cytometry is used to detect as well as measure the chemical and physical characteristics in the population of cells [5]. It is a laser based technology and which is also known as an optical technique. For measuring the physical and chemical characteristics of individual cell the flow cytometry uses the advantages of microscopy and biochemical analysis [10]. The main advantage of this technique is that it can measure several parameters simultaneously. A sample containing the cells is passed into the flow cytometer instrument. The laser beam is used as an incident beam and the sample containing the cell is passed through this laser beam. The light gets scattered from the beam gives the various characteristics of the cell. The Flow cytometry technique has wide applications in the study of eukaryotic cells. In Flow cytometry, a laser beam is used as the incident beam and reflected laser beam from the sample is measured, that is the scattering and fluorescence of laser beam is measured [11]. This technique has a main advantage that is it can collect number of output data from a single cell. The flow cytometry also has the capability to analyse thousands of cells in one second depending on the concentration of cell in sample and also the type of sample. This technique can be used for detecting microorganisms, protein engineering, cell sorting, cell counting

- *Enzyme Linked Immunosorbent Assay(ELISA)*

  Enzyme Linked Immunosorbent Assay is a serological diagnostic technique which is used for detecting plant pathogens [12]. Elisa is also used for detecting virus antigens which is present in the tissue parts [13]. The microplate method of Elisa is an efficient technique for the detection of virus in a low virus concentrated condition. Elisa technique is a simpler and cheaper method which can be used for the processing of different types of samples. The methods of Elisa techniques are such that the antigens from the sample are collected and attached to the surface. Then an antibody which matches to this antigen is applied over the surface and the binding occurs between antigen and antibody. Then the antibody is linked to an enzyme and an enzyme substrate is added. The reaction produces color change in substrate.

- *Immunofluorescence (IF)*

  Immunofluorescence method is an optical microscopic technique and which is able to analyze microbiological sample [5]. It is also called as a cell imaging technology which uses antibodies to label specific antigen with a fluorescent die. Immunological methods are used for onsite detection because it requires only simple machines to detect monoclonal antibodies [14]. Immunofluorescence has a main advantage that it requires only limited microbial training which can be done at low cost. There are two methods of immunofluorescence that is direct and indirect Immunofluorescence. The difference between these two methods is that the direct Immunofluorescence uses single antibody and the indirect Immunofluorescence uses two antibodies. In direct Immunofluorescence method only primary antibody is participated where as in indirect method both the primary and secondary antibodies are present. The Immunofluorescence can also be used to detect the infections in plant tissues which are caused by the pathogens. This technique can be used for individual cells/ cluster of cells and also on tissue section.

ISSN(Online): 2320-9801
ISSN (Print) : 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Website: www.ijircce.com

Vol. 7, Issue 3, March 2019

B. *MOLECULAR METHODS*

Molecular methods used to identify the plant pathogenic fungi. Molecular methods can be used for the study of genetic variability in the populations of pathogens. The high degree of specificity of the molecular method helps to distinguish between organisms at different taxonomic levels [7].

- *Fluorescence in situ hybridization (FISH)*

  Situ hybridization is a procedure that allows to locate the position of specific DNA in a chromosome. Cytogenetics, which is a study of chromosomal structure and function has a wide application of situ hybridization. Most situ hybridization procedures use fluorescent probes to detect the DNA sequences. This process is commonly referred as Fluorescencein situhybridization (FISH) [27]. FISH is a powerful tool for the detection of plant pathogens. FISH allows a direct visualization of the hybridization sites and also several probes can be detected simultaneously with different fluorochrome [28]. In FISH, the first step is to make a fluorescent copy of probe sequence or to make a modified copy of probe sequence. The copies have to be rendered fluorescent later. The target and probe sequence should be denatured using chemicals or heat before the hybridization. Denatured probes are able to form hydrogen bonds between target and probe which facilitates the process in further hybridization processes. The probe and target are then mixed up and probe is specifically hybridized to its complementary sequence on chromosome. The hybridization process is visualized by using fluorescent. The sensitivity and resolution should lie under the technical limit of fluorescent microscopy [27].

- *Polymerased Chain Reaction (PCR)*

  The Polymerase Chain Reaction is a laboratory technique which provides a simple method for the exponential amplification of specific DNA sequences through DNA synthesis [24]. The three essential steps of PCR are Melting of the target DNA, Annealing of two oligonucleotide primers to the denatured DNA strands, and Primer extension by a thermostable DNA polymerase. These three steps are done at different temperatures [25]. The specificity of the PCR is derived from the synthetic oligonucleotide primers. By the introduction of Thermusaquaticus (Taq) DNA polymerase, the use of PCR had a rapid growth in plant pathology. Due to the enzyme's relative stability at DNA-meltintgemperatures, it is capable to eliminate the need of enzyme replenishment after each cycle of synthesis. This reduces PCR costs and allows automated thermal cycling. Short pieces of single-stranded DNA is used as the PCR primer [26]. Random Amplified Polymorphic DNA (RAPD) finger printing uses PCR and a set of short, random-sequence oligonucleotide primers that produce characteristic profiles of amplified products for each organism. Since, the DNA of plants and other organisms are amplified using random primers, plant pathogens have to be first purified or cultured from their hosts to obtain finger prints. Hence RAPD analysis cannot be used for direct detection in plant pathogens [24]. In short PCR allows detection of target sequence in complex specimens without culturing. The sensitivity, speed and adaptation to different varieties make them wide acceptance in plant pathology as well as many fields of biology.

- *DNA Arrays*

  DNA microarray is also known as biochip which consist of collection of microscopic DNA spots. These micro arrays provide the study of genes inside the cells and clusters. They are used for the detection of microbial pathogens. The RNA from the samples are isolated and measured instead of isolating the DNA from sample. Arrays are classified into two. That is arrays of spot size 200 µm in diameters are called micro arrays and that with size 300 µm in diameters is called macro arrays. The micro array contains low density arrays and macro contains the high density arrays. The DNA arrays has the ability to display the expression of thousands of genes simultaneously, thus it is considered as a powerful tool for genetic analysis [17].

V. INDIRECT DETECTION METHODSN

Indirect method, instead of identifying the pathogens is detected. It is also called as a non-invasive method of plant disease detection. Indirect method uses various parameters like change in temperature, rate of change to transpiration rate, morphological change for identifying plant disease [20]. Imaging and spectroscopic techniques are commonly used indirect methods.

A. *IMAGING TECHNIQUES*

The imaging techniques involves the Hyperspectral imaging and fluorescence imaging. These methods are used to analyse the morphological changes that are encountered in the pathogenic plants. The morphological changes may involve change in colour and structure which are extracted using optical sensors. These changes can be evaluated using various image processing techniques.

- *Hyperspectral Imaging*

  Hyperspectral Imaging is another imaging from the Electromagnetic spectrum for its processing. The Hyperspectral Imaging technique is more advanced than the multispectral technique, that it has a number of spectral bands (in terms of the quality and quantity of information available). In Hyperspectral Imaging technique, the reflected light from the plants are measured along a number of bands across the Electromagnetic spectrum which is represented as a hypercube [20]. In this, spectral reflectance from each pixel of an image is obtained. (That spectral reflectance will be within the range of the spectrum). The information contained in a hyper spherical image is based on the spectral axis and spatial axis [21]. There are two spatial axes, that is X axis and Y axis and the spectral axis is the Z axis [5].

- *Fluorescence Imaging*

  In fluorescence imaging an incident beam of light is made to fall on the leaves in order to measure the chlorophyll fluorescence and the changes caused in the parameters of fluorescence is used for analyzing the pathogen infections [5]**.** The UV lights are used as incident light for imaging the chlorophyll fluorescence [22].

B. *SPECTROSCOPY TECHNIQUES*

Spectroscopy is the study of the interpretation of electromagnetic waves such as ultraviolet, infrared and visible rays. Spectroscopy technique is a non-invasive technique used for the detection of specific type of diseases. In spectroscopy the radiant intensity is measured which is the function of wavelength. An absorption or emission spectrum is used for describing the spectroscopy data. Two types of spectroscopy are fluorescence spectroscopy and visible/near infrared spectroscopy.

- *Fluorescence Spectroscopy*

  It is otherwise known as spectrofluorometric which is a type of spectroscopic methods. It is used to analyse the fluorescence from the samples. This technique uses a beam of light for exciting the electrons present in the compounds and which causes the electrons to emit light. There are mainly two types of fluorescence that are produced by green leaves, they are: Blue – Green fluorescence in the range of about 400-600 nm and Chlorophyll fluorescence in the range of 650-800 nm [18]. In plants the fluorescence emissions can be divided into two. That is emissions from leaf epidermis and emissions from leaf veins [19]. There are various factors which gives the ratio between the blue-green fluorescence and chlorophyll fluorescence, they are plant type, exposure to sunlight and proportion of fluorescence absorbing materials in the leaf. Sensors can be used for the fluorescence sensing in plants which will be a portable sensor. The fluorescence mainly consists LED Unit and photo diodes. The LED unit provides the excitation wavelengths and the photodiodes are used for measuring the fluorescence emissions from the leaf. The fluorescence sensor is taken directly to the field to measure the spectral data. Different methods like Principle Component Analysis (PCA) or neural

ISSN(Online): 2320-9801
ISSN (Print) : 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Website: www.ijircce.com

Vol. 7, Issue 3, March 2019

network based classification algorithms can be used to analyze the results of fluorescence spectroscopy. For the classification of fluorescence spectrometric results with more than two classes, the cluster analysis and Partial Least Square (PLS) regression can also be used.

- *Visible / Near Infrared spectroscopy*

  Visible / Near infrared spectroscopy is sensor technology which is applied for the detection of the presence of diseases or for the detection of parameters like stress, quality etc. [23]. VIS/NIR spectroscopy gives a detailed spectral information based on the interaction of the physical or chemical constituents in the plants with the light. Usually 400 to 2500 nm spectral range is utilized by VIS/NIR spectroscopy where 400 to 700 nm is the VIS region and 700 to 2500 is NIR region.

VI. CONCLUSION

Plant disease detection is a key method for the reduction of agricultural losses. Detecting diseases in an early stage is very useful for preventing the growth of the pests and also the spread of diseases, thereby reducing use of pesticides in the agricultural field. The review was about the detection of diseases caused to plants by various pathogens. It involved both the direct and indirect methods of plants disease detection. The direct method consists of serological and molecular techniques whereas the indirect method deals with the imaging and spectroscopic techniques. The direct methods require experts for its operations and also they all are time consuming. The difficulty in the serological methods like ELISA, flow cytometry, Immunofluorescence for plant virus detection is in the production of good virus specific antiserum. The molecular techniques require an elaborate procedure during the preparation of samples and also they give only reliably accurate results. The serological and molecular methods require high throughput analysis in a large number of samples. The Direct methods can provide better accuracy than indirect methods. Even though there are some dilemma. it requires pre-processing which may cost a couple of days. whereas the Indirect methods identify plant pathogens instantly by extraction of features like morphological changes, temperature change and by analysis of organic compounds released by infected plants. The method uses optical image sensors which provide detailed information based on electromagnetic spectrum and hence the prediction of diseases. The spectroscopy techniques and imaging techniques are the popular indirect methods. The spectroscopy methods use the absorption or emission spectrum for describing the spectroscopy data. In Hyperspectral imaging the entire spectrum is acquired at each point. These two methods support on-field testing for the disease detection.

REFERENCES

1. 1. Jasmeet Kaur, et al. “A review paper on plant disease detection using image processing and neural network approach.” International Journal of Engineering Sciences & Research Technology (April 2016).
2. Golhani, Kamlesh, et al. "A review of neural networks in plant disease detection using hyperspectral data." Information Processing in Agriculture (2018).
3. Ferentinos, Konstantinos P. "Deep learning models for plant disease detection and diagnosis." Computers and Electronics in Agriculture 145 (2018): 311-318.
4. Dr. P.V. Rama Raju, et al. “Leaf disease identification and classification using k-means clustering.” Global Journal of Engineering Science and Researches(June 2018).
5. Fang, Yi, and RamarajaRamasamy. "Current and prospective methods for plant disease detection." Biosensors 5.3 (2015): 537-561.
6. Lima, J. Albersio A., et al. "Serology applied to plant virology." Serological diagnosis of certain human, animal and plant diseases. IntechOpen, 2012.
7. Capote, Nieves, et al. "Molecular tools for detection of plant pathogenic fungi and fungicide resistance." Plant pathology. IntechOpen, 2012.
8. Sankaran, Sindhuja, et al. "A review of advanced techniques for detecting plant diseases." Computers and Electronics in Agriculture 72.1 (2010): 1-13.
9. Balodi, Rekha, et al. "Plant disease diagnosis: Technological advancements and challenges." Indian Phytopath 70 (2017): 275-281.
10. Diaper, J. P., and C. Edwards. "Flow cytometric detection of viable bacteria in compost." FEMS microbiology ecology 14.3 (1994): 213-220.
11. Chitarra, Luiz G., and Ruud W. Van Den Bulk. "The application of flow cytometry and fluorescent probe technology for detection and assessment of viability of plant pathogenic bacteria." European journal of plant pathology 109.5 (2003): 407-417.
12. M.L. EDWARDS and J.I. COOPER. “Plant virus detection using a new form of indirect elisa.” Journal ofVirologicalMethods, 11 (1985) 309-319, Elsevier.

ISSN(Online): 2320-9801
ISSN (Print) : 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Website: www.ijircce.com

Vol. 7, Issue 3, March 2019

13. Clark, Michael F., and A. N. Adams. "Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses." Journal of general virology 34.3 (1977): 475-483.
14. Gao, L., et al. "An immunofluorescence assay for the detection of wheat rust species using monoclonal antibody against urediniospores of Pucciniatriticina." Journal of applied microbiology 115.4 (2013): 1023-1028.
15. Puri, S., I. K. Tiwari, and R. K. Saraf. "Role of polymerase chain reaction in plant pathology." Int J Sci Nat 6 (2015): 115-118.
16. Capote, Nieves, et al. "Molecular tools for detection of plant pathogenic fungi and fungicide resistance." Plant pathology. IntechOpen, 2012.
17. Hadidi, A., H. Czosnek, and M. Barba. "DNA microarrays and their potential applications for the detection of plant viruses, viroids, and phytoplasmas." Journal of Plant Pathology (2004): 97-104.
18. Sankaran, Sindhuja, et al. "A review of advanced techniques for detecting plant diseases." Computers and Electronics in Agriculture 72.1 (2010): 1-13.
19. Sankaran, S., and R. Ehsani. "Detection of huanglongbing disease in citrus using fluorescence spectroscopy." Transactions of the ASABE 55.1 (2012): 313-320.
20. Moghadam, Peyman, et al. "Plant disease detection using hyperspectral imaging." 2017 International Conference on Digital Image Computing: Techniques and Applications (DICTA). IEEE, 2017.
21. Mahlein, Anne-Katrin, et al. "Recent advances in sensing plant diseases for precision crop protection." *European Journal of Plant Pathology* 133.1 (2012): 197-209.
22. Chaerle, Laury, and Dominique Van DerStraeten. "Imaging techniques and the early detection of plant stress." Trends in plant science 5.11 (2000): 495-501.
23. Abu-Khalaf, N., and M. Salman. "Detecting plant diseases using visible/near infrared spectroscopy." NIR news 24.4 (2013): 12-25.
24. Henson, Joan M., and Roy French. "The polymerase chain reaction and plant disease diagnosis." Annual review of phytopathology 31.1 (1993): 81-109.
25. https://nptel.ac.in/courses/102103017/pdf/lecture%2037.pdf
26. https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/a/polymerase-chain-reaction-pcr.
27. https://www.nature.com/scitable/topicpage/fluorescence-in-situ-hybridization-fish-327
28. Heslop-Harrison, Pat, et al. "Fluorescent in situ hybridization of plant chromosomes: illuminating the Musa genome." INIBAP annual report (1998): 26-29.
29. Khaled, AlfadhlYahya, et al. "Early detection of diseases in plant tissue using spectroscopy–applications and limitations." *Applied Spectroscopy Reviews* 53.1 (2018): 36-64.