Imran Ul Haq
Siddra Ijaz *Editors*

Trends in Plant Disease Assessment

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Preface

Disease assessment is an essential aspect of the plant disease epidemics study. Phytopathometry is the foundation for all subsequent analyses, interpretations, characterization, and comparisons of plant disease epidemics. Disease quantification represents the magnitudes of the effectiveness of management strategies adopted in controlling plant diseases and also provides numerical estimates of crop losses due to these diseases. The accuracy in plant disease assessment is the pillar of integrated disease management. An assessment in a precise way to represent a quantitative link between theory and practice of disease management. If someone wants to develop a predictive model in a meaningful way, disease intensity must be quantified with a high degree of accuracy. Therefore, we are attempting to provide the readers with comprehensive information in a well-compiled way. Any manuscript reflects the authors' specialty, interest, and training. As the title of this manuscript depicts, it deals with plant disease assessment approaches. In this manuscript, a broad topic of study, "Plant diseases assessment," has tried to break down into different striking chapters. It will give a comprehensive picture of approaches to visual estimation for assessing plant diseases.

This book will help plant scientists directly or indirectly deal with plant disease diagnosis and management. It will be helpful for researchers and students in gaining knowledge and skills in disease quantification, developing predictive models for plant disease epidemics, assessing crop losses, and the magnitude of plant disease control methods. This book deals with the classical concept of plant disease assessment and methods based on visual observations to knowledge regarding the modern and emerging technologies in phytopathometry, predictive models, disease warning systems, and decision support systems. It will delineate remote sensing approaches for assessing diseases in plants. Here, we squeeze it with a statement that this manuscript gives a beautiful shift from classical to modern approaches to explaining plant disease assessment.

Faisalabad, Pakistan

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About the Editors

Imran Ul Haq with a bright career in agriculture, plant pathology, and fungal molecular biology, had Post Doc from the University of California Davis, USA. He is currently serving as an Associate Professor in the Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan. He has supervised more than 40 graduate students and established the Fungal Molecular Biology Laboratory Culture Collection (FMB-CC-UAF), an affiliated member of the World Federation for Culture Collections (WFCC). He has published more than 50 research articles, 6 books, 4 laboratory manuals, and several book chapters. He has made colossal contributions to fungal taxonomy by reporting novel fungal pathogen species in plants. His research interests are fungal molecular taxonomy and nanotechnology integration with other control strategies for sustainable plant disease management.

Siddra Ijaz with a vibrant career in agriculture and biotechnology, had a Post Doc from the Plant Reproductive Biology Laboratory, University of California Davis, USA. She is currently serving as an Associate Professor in the Center of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan. She is also serving as Deputy Managing Editor of an international impact factored Journal. She has supervised more than 50 M.Phil and Ph.D. students. She has published more than 50 research articles, 7 books, and several book chapters. Her research focus includes plant genome engineering using transgenic technologies, genome editing through CRISPR/Cas systems, nanobiotechnology, and exploration of genetic pathways in plant–fungus interactions.

Phytopathometry: A Transdisciplinary Concept

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Imran Ul Haq and Siddra Ijaz

Abstract

Globalization, modern cultivation techniques, climate change, and human activities have promoted the distribution of plant pathogens, resulting in frequent host–pathogen interactions and disease incidences. These causative factors are impossible to control as even the most rigorous quarantine system could not completely avoid the movement of plant pathogens and germplasm across countries and continents. Susceptibility of cultivated varieties against plant pathogens, especially fungi, has significantly increased because varieties are developed focusing on higher yield. Additionally, plant pathogens undergo frequent mutations and genetic changes to adapt to climate changes, overcome pesticide resistance, and infect plant germplasm previously resistant. Plant disease identification, quantification, and estimation of subsequent yield losses are crucial in modern-day agriculture to ensure food safety and security for the increasing global population. Phytopathometry utilizes systematized and specialized approaches for plant disease assessment and presents qualitative and quantitative data. Phytopathometry underpins all activities in plant pathology and extends into other related disciplines such as agronomy, plant breeding, and horticulture. Digital and biotechnology underpinned by contemporary artificial intelligence efficiently process sensory data for plant disease measurement. Modern phytopathometry tools aided with detailed knowledge of pathogen–host system biology are poised to become an integral part of precision agriculture.

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Use of machine learning, deep learning, digital technology, biotechnology, engineering, and nanotechnology in phytopathometry is exposing plant pathologists to new terminology, concepts, and ideas which were not even thinkable a few decades ago. Moreover, innovations in robotics have provided flexibility and precision in deploying these sensors for accurate disease assessment, even in large field areas. In this chapter, we have discussed various phytopathometry tools and approaches and their transdisciplinary uses.

Keywords

Disease index · Visual assessment · Remote sensing · Digital imagery · Artificial intelligence · Advanced technology

1.1 Introduction

Phytopathometry is the branch of plant pathology that utilizes systematized and specialized approaches to assess and measure plant diseases. This neologism was first coined by Large (1953) in his research article on late blight of potato and choke of cocksfoot diseases. He researched acquiring valuable data/information from disease surveys based on the new science of phytopathometry (Large 1953). Phytopathometry was derived from the Greek words "phyton" plant, "pathos" disease, and "metron" measure. Phytopathometry is a ""branch of phytopathology that deals with detection, identification, and measurement of plant diseases indicated by disease symptoms or pathogen signs on particular disease sample/s."

Gregory (1982) recapitulated E.C. Large's disease measurement requirements focusing on resulting yield losses: morphological characteristics and development of healthy crop plants; disease path and trajectory on field plants; evaluation of disease intensity by devising/utilizing standard area diagram or field key; assessment of disease progression in field trials over the course of several years and comparing yield with control plots; assessment of disease severity concerning particular host growth stage and its effect on yield by using disease progress curves. This tactical and expanded perspective of phytopathometry, stated by E.C. Large and reiterated by P.H. Gregory, encompasses yield losses on disease (Gregory 1982; Nutter et al. 1991; Large 1966). However, phytopathometry should not be considered synonymous with an estimation of plant disease losses because it is a broader term aiming to ameliorate the recording and reporting of plant diseases by presenting qualitative and quantitative results. This encompassing approach makes phytopathometry a transdisciplinary concept as it helps in forecasting yield losses (agronomy), breeding aiming for resistance against plant diseases (plant breeding and genetics), evaluation of disease control methods and their application time, system biology and co-evolution of host–pathogen interaction (biotechnology), and myriad other reasons. Hence, phytopathometry underpins all plant pathology activities and extends into other related disciplines. Artificial intelligence (AI) and digital

technology have recently been used to design automated sensors in phytopathometry (Madden et al. 2007b; Bock et al. 2010, 2016).

It is worth mentioning that phytopathometry measures plant diseases but is not limited or subservient to disease epidemiology or crop yield losses as its services intersect with statistics and measurement science and also interface closely with visual disease assessment and imaging and sensing technology. Historically, visual disease estimation was standard as compared to instrument-based disease measurement. Recent advances in imaging and sensing technology have significantly improved and opened new horizons for remote phytopathometry, especially in achieving the goals of plant disease quantification. This sensor-based and remote sensing approach in phytopathometry is transdisciplinary as it involves plant pathology, engineering, agronomy, and information technology (Bock et al. 2021).

1.2 Role of Phytopathometry in Precision Agriculture

Globalization, recent cultivation techniques, climate changes, and human activities promoted the distribution of plant pathogens and resulted in frequent plant–pathogen interactions and disease incidences. These causative factors are almost impossible to control, and the most rigorous system of quarantine and preventive measures could not completely avoid the movement of pathogens and plant germplasm across continents. Susceptibility of plants against pathogens, especially fungi, has increased in modern cultivation practices because crop varieties are developed focusing on higher yield. These varieties, as compared to wild relatives, are less adaptive to local conditions and less resistant to pathogens. Additionally, pathogens have undergone genetic changes to adapt to climatic changes and overcome plant and pesticide resistance. New pathogenic strains have emerged, posing new challenges to sustainable agriculture. Cultivation of genetically uniform crop plants, unbalanced fertilizers and pesticides, reduced crop rotation, and low tillage cultivation may further aggravate the crop yield losses. With new challenges to crop production, demand for innovative strategies to identify and control plant pathogens has also increased. Plant pathogens compromise the quality, quantity, and input costs, seriously threatening world food safety and security. For practical crop production, balanced approaches to integrated plant protection are being proposed by researchers. These approaches recommend crop rotation, resistant verities, effective chemical and biological plant protection strategies, accurate estimation of disease severity, and monitoring of the spread of plant pathogens (Parnell et al. 2017; Oerke and Dehne 2004; Gewin 2003; Fisher et al. 2012; Brasier 2008; Ul Haq and Ijaz 2020; Ul Haq et al. 2020).

1.3 Phytopathometry Tools in the Modern Era

Today, visual disease assessment based on scientific reasoning and understanding provides a firm base to reliably and accurately assess plant diseases, i.e., standard area diagrams (Del Ponte et al. 2017) and ordinal disease scale (Chiang et al. 2014). However, digital technology underpinned by contemporary artificial intelligence incredibly processes sensor data for measuring disease severity. Image analysis, thermal imaging, and chlorophyll fluorescence are innovative and practical assets of disease measurement. Innovations in robotics have provided flexibility and precision in the application of these assets. Hence, digital technologies are poised to become an integral part of precision agriculture. AI has been applied to plant pathometry, though recently, and is crucial for the field success of ingeniously designed sensors. The use of AI and digital technology in phytopathometry exposes plant pathologists to new concepts, terms, and ideas that were not even thinkable a few decades ago (Bock et al. 2022).

1.4 Phytopathometry Approaches

Disease quantification by symptomatology falls under the umbrella term "remote sensing." Remote sensing could be defined as gathering and monitoring the physical information about an area/object by measuring the radiation (reflected/emitted) without direct contact. Various methods are used to detect, identify, and quantify the symptoms such as thermograph, laser induced fluorescence, nuclear magnetic resonance imaging, radar, microwave, and cameras. Visual estimation and image analysis technologies fall under remote sensing (Abazov et al. 2006).

Disease severity is determined by analyzing the digital image of a given sample. It could be inferred by the disease-affected area, and the texture and color of the infected area by using the segmentation step are quantification algorithms which isolate the affected area from healthy plant parts and process. Disease severity analysis based on symptoms, even if performed by plant pathologists who follow the complete diagnostic guidelines, results in variability or error due to some degree of subjectivity. This statement means that methods employed to validate the automatic approaches to disease quantification are also subject to some degree of variability. It should be considered when evaluating the performance of remote sensing methods (Arnal Barbedo 2013; Merga 2018).

1.4.1 Visual Disease Assessment

In visual assessment, the eye and the relevant parts of the brain, in combination, work rapidly to acquire an image, process and interpret it, and act as an image analysis system. This quick process helps the observer to assess the disease type and severity of a particular plant sample. The observer's response regarding detection and disease estimation depends upon the visual rater's cognitive ability, eye health,

and how light/color is perceived (Bock et al. 2010). Various disease assessment scales are used in visual phytopathometry:

1.4.1.1 Nominal Scales

These scales differentiate disease stages through simple descriptive terms such as "mild," "moderate," or "severe." These scales are subjective and lack quantitative information. These scales are used only when the observer performs the task at a particular location and in a specific season (Bock et al. 2021; Nutter et al. 1991).

1.4.1.2 Ordinal Scales

Ordinal scales are widely used while rating disease severity for viral diseases as symptoms are difficult to measure quantitatively (Madden et al. 2007a; Bock et al. 2010). These disease severity scales are also descriptive, simple, and have use for a particular observer, location, and season. These scales divide disease severity stages into different classes which represent the increasing disease symptoms severity. These scales are further explained by different diagrams and mostly in descriptions explaining symptoms intensity. Sometimes, mostly in case of fungal diseases, these grade fungal disease severity divides the symptoms into numerals, e.g., ranging from 0 to 9. These are also used in disease resistance breeding programs while assessing genotypes.

1.4.1.3 Interval Scales

Disease interval scales, also known as category scales, comprise different categories that contain numerals representing disease severity in percentage. These scales include a standard area diagram of different levels, e.g., 1–5 for rust diseases, illustrating symptoms severity of 1%, 5%, 10%, 20%, and 50%. Hence, the sample leaf is assigned a specific category by the observer.

1.4.1.4 Ratio Scales

The ratio scale is a percent scale and has been used in phytopathometry over the years for studying various diseases. In this scale, the observer has a continuous range of percentages from 0% to 100% and grades the sample leaf according to the percentage area covered by the disease symptoms. Observers, according to their ability, vary in accuracy and reliability while assessing disease severity.

1.4.1.5 Advantages and Disadvantages of Visual Disease Assessment

Visual disease assessment is quick. Rater's ability to identify and differentiate between different diseases could be enhanced with some training. Rater can choose from various available disease rating scales and can assess disease severity without any equipment. Visual assessment accuracy depends only on the observer's ability, health, and concentration. This subjectivity results in significant variability in disease assessment. However, visual inspection is used by farmers and plant pathologists to assess the disease for the foreseeable and more appropriate disease control strategy. Other remote sensing and phytopathometry approaches are making significant contributions.

1.4.2 Digital Imaging in Phytopathometry

Visual monitoring is a widely used method in plant disease detection and, if combined in a prognosis system with regional weather and other epidemiological parameters, can help forecast disease spread in specific regions. It, especially the moisture and temperature data, could help in timely and effective plant protection strategies to avoid the upcoming disease threat in fields or greenhouses (Bock et al. 2010). However, visual disease assessment by an expert in the area is time-consuming and requires an observer, which is a bottleneck in disease management, especially when monitoring large fields. Hence, various other innovative methods are being developed to estimate and accordingly adapt disease control strategies effectively.

Disease severity plays a crucial role in understanding the disease development and consequent losses in various pathosystems but is challenging to achieve in visual and sensory-basedphytopathometry approaches. Since the first visual assessment attempt of disease quantification by Cobb in 1892, it has significantly improved and is better understood (Cobb 1892). Though instruments, cameras, and aerial photography, were used in the first half of the twentieth century (Bawden 1933) but usage of proximal tools for remote sensing was performed and reported in the late twentieth century, i.e., thermal spectrometers (Pinter et al. 1979), image analysis (Lindow and Webb 1983), and multispectral radiometer (Nutter et al. 1985). The nascent remote sensing field owing to sophisticated sensors is significantly effective in identifying, detecting, and measuring the disease (Mahlein et al. 2018; Bock et al. 2022).

1.4.2.1 Principles of Photography

Photography has been used in phytopathometry for many decades. Aerial photography started in the 1920s still and continue to use to detect and quantify plant disease severity. Microscopic digital image analysis is widely used in plant pathology to study pathogen's morphology. Digital cameras are inexpensive and commonly used in laboratories and fields. These are the primary device while sampling and are easy to use, and the rater could study the disease symptoms later. Digital cameras include a light-sensitive screen (which captures the light from the image), a lens (increase magnification), and a display screen. Monochromatic cameras measure the intensity of the incoming light with photo sensors on their screen. Photo sensors convert captured light into electric charge and release proportional to the received light intensity. Photo sensors are memory cells whose contents, analog signals, are then converted into binary signals (0, 1) with the help of a frame grabber, and the binary data is transmitted to the computer. Computer based on these binary digital readings of 0 and 1 draws the image on the screen. So, each photo sensor on a digital camera's screen is represented on the computer screen by a pixel whose location and brightness are determined through image processing. On their photosensitive screen, color cameras have specific photo sensors for red, green, and blue. These photo sensors could be re-arranged in different ways. Each photo sensor is sensitive to particular wavelength/color, and the final image is prepared by advanced image analysis, which compares each pixel with its surrounding pixels using an interpolation

algorithm. This algorithm portrays the original color of the sample by using the RGB color model.

1.4.2.2 Factors Responsible for Image Quality

Various factors that affect digital image quality, such as focus, light on the object, and light uniformity, are affected by image analysis.

1.4.2.3 Pros and Cons of Digital Photography

Image analysis systems with agreement verification are quick, accurate, reliable, powerful, and relatively inexpensive. According to needs and issues, specific software is adapted in phytopathometry. However, there is tremendous variability in plant color, shape, texture, and disease symptoms which influence the performance and results of image analysis. Software should deal with various diseases on various plants and varying physiological conditions or damage on sample leaves. Hence, program training and ground trothing are done to make it more efficient and ensure quality disease severity measurement results.

1.4.3 Android Applications to Quantify Plant Disease Severity

Android portable applications are new tools for semiautomatic disease assessment. These are used for individual leaves and other samples. These applications, such as "Leaf Doctor," use an algorithm to accurately, precisely, and robustly identify leaf shapes, lesion types, and disease severity. The accuracy of Leaf Doctor has been evaluated in various research articles, and it was found to have a low degree of error; however, in some cases, such as mallow rust and lilac powdery mildew, due to high color contrast between diseased and healthy leaf sections. Compromising contrast results in reduced accuracy and robustness of the Leaf Doctor Algorithm, such as in powdery mildew of Lilac (Barbedo 2014). Coefficient variation shows a significantly negative relationship (both linear and nonlinear) with mean observed disease severity, which is also observed in individual raters estimating disease severity manually. These applications are accurate, precise, and robust for most diseases with faster processing time. This application also helps rapidly construct standard area diagrams from actual host–pathogen systems, estimated by an expert. These SADs improve the accuracy and precision of estimates with excellent reproducibility (Braido et al. 2014; Pethybridge and Nelson 2015).

1.4.3.1 Image Processing Software

Image processing software allows the editing of the obtained image in various ways, i.e., contrast correction, image rotation, sharpness, inversion, and various other manipulations. It is achieved by using multiple image-enhancing software such as Adobe Photoshop. Image editing, modification, geometric correction, and edge enhancement are also performed using image analysis programs (Bock et al. 2010; Merga 2018). Specific image analysis protocols must be followed regardless of the software being explored. The color image is composed of red, green, and blue, and

each image pixel has a specific value for each respective color based on the RGB model. This model generates the correct color in a three-dimensional color space.

1.4.4 Statistical Methods in Phytopathometry

Statistical models are used to analyze the false positives and negatives in image analysis by comparing them with visual observations of an expert. Statistical methods evaluate the quality of image analysis results compared with the estimated actual value for assessing disease severity. Moreover, the repeatability and reproducibility of various image analysis methods and statistical methods are explored (Zhao et al. 2009; Bock and Nutter 2011).

1.4.4.1 Regression Analysis

Regression analysis is widely used in image analysis to evaluate the quality of disease severity measurements. It judges the accuracy, precision, and reliability of results; however, it should be applied carefully as specific circumstances could generate incorrect conclusions. Standard errors, slope, and intercept are regression parameters assessing the estimated measurements' quality. Reliability and precision are measured using the coefficient of determination; its higher value indicates high precision. Various color and monochrome images of different disease samples assessed through image analysis were evaluated for accuracy and precision using a regression model.

1.4.4.2 Concordance Correlation Coefficient

Lin's concordance correlation coefficient quantifies agreement in disease severity by evaluating the degree to which the observations fall on the concordance line. It combines the systematic and constant bias measure of accuracy with precision to consider the relational fit to the concordance line. It was used in image analysis of citrus canker symptoms severity to investigate the precision and accuracy of results (Bock and Nutter 2011).

1.4.4.3 Analysis of Variance and General Linear Modeling

ANOVA and GLM investigate the source of error in disease severity estimates. Another method, Kruskal–Wallis test, compared the image analysis and visual assessments of powdery mildew on cherry leaves and found that image analysis produced significantly negative results compared to visual rating.

1.4.4.4 Correlation Coefficient

The correlation coefficient indicates the degree of precision in disease severity assessment obtained using various methods. It quantifies the strength between variables and measures it by working as a component of the concordance correlation coefficient, i.e., strawberry Phomopsis disease severity rating methods and citrus canker severity on grapefruit. Hence, it evaluates and compares various image analysis methods and raters. Bland–Altman plots, absolute error, relative error,

and chi-square hypothetical variance tests are also used to assess the precision of image analysis by comparing with visual assessments.

1.5 Hyperspectral Imaging

Optical sensors operate within different regions of the electromagnetic spectrum to measure the changes in plant physiology either due to abiotic stress or due to the disease. Biotic or abiotic diseases cause changes in tissue color, canopy morphology, leaf shape, plant density, transpiration rate, and interaction of radiation with plant surface; these variations could be measured using highly sensitive and powerful sensors (Fiorani et al. 2012; Mahlein 2016). These sensors are noninvasive and provide information beyond the visible light spectrum, except RGB imaging, which expands human perception and phytopathometry capabilities. Hyperspectral sensing showed promising results in identifying various plant parameters and diseases. It enables the detection of biotic diseases and abiotic stresses, resulting in new opportunities for field phenotyping and disease management.

1.5.1 Spectral Resolution Range of Hyperspectral Sensors

Hyperspectral sensors have increased spectral resolution compared to conventional (RGB) digital cameras. These can assess the RGB, visible spectrum (400–700 nm), near-infrared spectrum (700–1000 nm), and shortwave infrared (1000–2500 nm). Hence, the modern hyperspectral sensor covers high complexity spectral range from 350 to 2500 nm. These sensors are categorized into various classes based on their respective spectral resolution, scale, and imaging principles. Spectral resolution depends on measured wave bands; spectral scale could be ultraviolet, visible, near-infrared, or shortwave infrared, whereas imaging principle could be based on imaging or non-imaging system.

In the case of the non-imaging sensor system, spectral information over a specific area and within the sensor's field of view is averaged with spatial resolution information. Hyperspectral data is observed as three dimensions in huge matrices with spatial *x*-axes, *y*-axes, and on *z*-axes, spectral information depicting intensity/wave band is represented. Due to spatial dimension, HSI systems provide extra details such as color, gradient, and shape and are preferred over non-imaging methods (Behmann et al. 2015). Though multispectral sensors are comparable to HS sensors but are less complex and informative and provide object's spectral information in broad wave bands such as RGD and NIR. These are less expensive and are used in unmanned airborne vehicles due to their lightweight. The reflectance average of non-imaging hyperspectral sensors provides spatial resolution with a specific allocation of the hyperspectral pixel. Research on HSI helped to design and develop new materials, efficient detectors, and software, and is widely used in various fields, e.g., food production, agriculture, and medicines (Cheng et al. 2017). Hyperspectral imaging provides site-specific, selective, and reliable information that

significantly improves sustainable agricultural management and is recommended and used in precision agriculture and plant phenotyping (assessing genotypes) (Simko et al. 2017).

1.5.2 Setup of Hyperspectral Sensors

Setups consist of a hyperspectral sensor, a light source (efficient in both sunlight and artificial light), and a control unit to measure and save the captured data (images). This setup could easily be mounted on any platform, such as tractors, unmanned airborne vehicles, robots, satellites, and handheld. The collected data from HSI is saved in a hyperspectral data cube, which displays spatial information data in a two-dimensional image. Wavelength range and targeted object determine the required imaging detector. The most commonly used sensors for visible and near-infrared spectra are complementary metal-oxide semiconductors and Si-based charge coupled devices, which are implemented on various sensing types, e.g., full-frame and push-broom cameras. Light interaction with the plant is the crucial knowledge required for hyperspectral analysis and interpretation of signals. The optical properties of the leaf depend upon light transmission through the leaf, light absorbed by the leaf, and light reflected (either from internal structure or from the waxy cuticle and cell wall). The maximum absorption and reflectance capacity of various compounds of the electromagnetic spectrum have been studied to explain the optical properties of plants (Mahlein et al. 2018; Merzlyak et al. 2008).

Several biochemical and histological changes could be characterized and interpreted during plant pathogenesis through HSI. Light reflectance from plant due to the involvement of various biophysical and biochemical interactions is a complex phenomenon. Visible light spectrum is mainly absorbed by leaf pigments such as chlorophyll, anthocyanins, and carotenoids (Gay et al. 2008). Near-infrared and shortwave infrared stimulate molecular motion and induce high reflectance and absorption by characteristic spectral pattern compounds. Leaf and cell structure influence the NIR reflectance due to matter interaction (Slaton et al. 2001), whereas SWIR can detect the plant/cell's water content (Seelig et al. 2008). Hence, remote sensing through hyperspectral imaging can determine the reflected and transmitted light and characterize the absorption activities of leaf compounds. Various android applications are being developed to determine the leaf compounds by observing reflected and transmitted light through HSI. However, due to its handling and setup complexity, transmittance sensing is used by only a few experts in phytopathometry.

1.5.3 Pathogenesis and Reflectance Signatures

Diseases are a gradual and dynamic process that consists of a series of events, one autonomously leading to the next, once pathogenesis is triggered. Pathogenesis triggers changes/abnormalities in various processes of the host plant's physiology and biochemistry. The resulting symptoms of pathogenesis influence the optical

properties of the infected host. We must dive further than identification based on characteristic symptomatology or visible signs of plant–pathogen interaction. Different plant–host interaction stages should be understood in more depth. Every plant pathologist knows that plant pathogens could be biotrophs (obtain nutrients from living tissues), necrotrophs (get nutrients from dying or dead tissues), or hemibiotrophs (could be both depending upon the developmental stage and environmental conditions). Each interaction consists of complex biochemical, biophysical, and histological processes regulating disease symptoms. Hence, reflectance changes during plant–pathogen interaction could be explained by abnormalities in the cell's chemical composition, ultimately generating changes in leaf appearance (e.g., chlorosis) and structure (e.g., lesions) during different stages of pathogenesis and at specific spatial and temporal dynamics host influence the reflective light's wavelength (Villa et al. 2013; Virlet et al. 2016).

Close-range HSI improves the spatial resolution of pathogenesis while considering specific space and time and results in more accurate hyperspectral analysis. The human eye and even the conventional HSI systems are challenged by deciding on early host–pathogen interactions when the size is observed in the submillimeter. This submillimeter size could be observed by improving the camera's spatial resolution through hyperspectral microscope setups, which could observe small and subtle reactions of the host plant. This ability opens up new opportunities for plant disease management, especially in breeding systems and compound testing. Hyperspectral image must be linked with the biological process involved in host–pathogen interaction. Consequently, an objective and accelerated plant phenotyping process could be achieved with reduced human and plant material involvement.

1.5.4 Potential of Hyperspectral Imaging in Phytopathometry

HIS, being a non-targeted remote sensing approach, collect a considerable amount of extra information about changes in processes and chemical composition of the host plant cell. This information is scattered across the measured spectrum, changing relevance over time (Behmann et al. 2015). Thus, analyzing the data to acquire a small proportion of specific information from the gathered hyperspectral signals is challenging. Recently, several approaches have been applied to improve HIS results for effective phytopathometry:

1.5.4.1 Preprocessing and Data Handling

Data is preprocessed to be normalized, having no illumination. In the case of sunlight as an illumination source, complex algorithms increase robustness against variation. This spectral normalization of data becomes critical if HIS involves extensive field observation data where continuous observations are impossible to make, and the interpolation strategy could fail. It is achieved by complex and robust approaches involving standard normal variant and explicit modeling by choosing suitable radiation transfer models. In an experiment, using the REGLOGSEP model based on singular value decomposition significantly improved the data quality, but

plant geometry was challenging (Jay et al. 2016). However, another model incorporating a radiative transfer model, PROCOSINE, was introduced for close-range scenarios, which can estimate local illumination and plant-physiological parameters (e.g., the content of plant pigments) and is less affected by plant geometry (Féret et al. 2017).

1.5.4.2 Vegetation Indices: An Important Tool in HSI

Hyperspectral experiment's massive data have various variable factors, but mostly only one is required for disease severity analysis. Vegetation indices highlight specific factors while reducing the impact of others (e.g., while assessing anthocyanins concentrations variable and unwanted chlorophyll content factor is suppressed by using anthocyanin reflective index). These express the spectral changes in a manageable manner. Though developed through remote sensing satellites, this technique is now being applied in proximal and close-range disease detection scenarios. In different disease studies, vegetation indices were successfully applied to deduce plant diseases, chlorophyll content, and yield estimation (Gitelson et al. 2014; Jay et al. 2017). However, vegetation indices do not cope with most bands, blank them out, and could not take full advantage of hyperspectral data.

1.5.5 Machine Learning

Machine learning approaches cope with hundreds of reflective bands and can detect, quantify, and characterize plant disease severity in laboratory and field conditions. Machine learning learns about model characteristics from the obtained data rather than using a specific model. These highly complex methods reveal the important unknown aspects of the hyperspectral data. Supervised approaches such as regression analysis rely on annotated training data, whereas unsupervised techniques don't. Unsupervised methods can recognize patterns because they can identify statistical coherences and uncover unknown relations in the data set. In a hyperspectral image analysis-based study to detect early Alternaria pathogenesis in oilseed crops, different machine learning algorithms were evaluated for their respective efficacy, and it was found that multilayer perception produced results with the highest accuracy (Baranowski et al. 2015). Zhu et al. evaluated the HSI machine learning algorithms for presymptomatic tobacco mosaic virus detection. Eight bands out of 434 variable bands were combined with texture features for evaluating machine learning algorithms. Three machine learning algorithms, namely neural network, random forest, and support vector machine, were assessed, and it was found that the neural network approach was able to detect tobacco mosaic virus, just after 2 days of inoculation, with high accuracy of 90% (Zhu et al. 2017). Powdery mildew of grapevine was detected by incorporating spatial and spectral characteristics with hyperspectral image analysis data. Various relevant wavelength bands in visible, near-infrared, and shortwave infrared spectrums were obtained using two cameras. When spatial context was integrated with HSI, it generated more informative hyperspectral images and increased the accuracy of results

compared with HIS data processing without incorporating spatial context (Knauer et al. 2017).

Unsupervised methods exploit all the available hyperspectral data but generate results with the target specification. Established data clustering methods are applied to hyperspectral data in preprocessing for segmentation and labeling. More advanced machine learning approaches could reparametrize the data informatively. Mixed techniques such as self-taught, semi-supervised, and transfer learning can deal with limited available information but are rarely used in phytopathometry.

1.5.6 Deep Learning

Deep learning represents the data more abstractly and informatively while optimized for a specific task. It has great potential for analyzing a large amount of hyperspectral data involving hundreds of bands and where particular optimal disease features are unknown (LeCun et al. 2015). Deep learning is a practical approach in HSI, where images have high dimensions with limited training data. HSI provides large amounts of data, complex features, and unknown and unfocused relations, which are beneficial for deep learning. In deep learning, specific programs are used to analyze the data on specialized servers. Resulted classification is sent to the smartphone and service providers by using the latest algorithms, detect and quantify the disease. This approach also improve the underlying model being used. For successful machine learning use in disease detection and quantification specific feature and model selection, optimization of data sets is required.

1.5.7 HSI for Disease Resistance Breeding

Continuous research in plant breeding for disease resistance is required because plant pathogens evolve to overcome resistant varieties and start pathogenesis. Due to their fast reproduction rate, it is a challenging process as plant pathogens mutate rapidly with varying impacts on growing varieties. Several sophisticated methods enable rapid screening of various genotypes for resistance against a particular pathogen. Plant phenotyping by a rater is done as, after several hybridizations and generations, a specific genotype could vary in its resistance against a pathogen in changing environmental conditions (Mahlein et al. 2018). Hence, when implemented in breeding programs, HSI improves the pace of breeding progress. Resistance could be qualitative or quantitative with or without observable changes. In a breeding experiment, a novel hyperspectral microscope evaluated resistance against powdery mildew for the barley. Gene-based vertical resistance mildew locus was responsible for hypersensitive response in the host plant, which was possible to retrospectively analyze hyperspectral features of the hypersensitive response in a particular space and time. It was possible because, after 5 days of inoculation, the response was visible as small brownish spots. Additionally, barley resistance response was detected using hyperspectral imaging 2–3 days before symptoms

appeared (Fiorani and Schurr 2013; Kuska et al. 2017). Type and amount of spectral properties along with temporal response are poorly understood for plant–pathogen resistance reaction. To screen unknown genotypes or new varieties, independent spectral patterns of particular resistance mechanisms are desirable.

1.5.8 HSI in Protected Horticulture

The increasing global population demand sustainable agricultural practices to fulfill its food demands. Protected horticulture by using greenhouses has proved effective in the judicious use of inputs and higher yields; however, diseases are a significant concern for growers in greenhouses as these can significantly limit profitability. Due to controlled and favorable conditions inside the greenhouse, fungal and bacterial pathogens can proliferate exponentially. Gummy stem blight in cucumber was automatically and accurately (95%) detected using point spectroscopy and spatial color imaging (Swinkels 2016). Hyperspectral line scan imaging was evaluated to assess the spinach growth in greenhouses. For this, induced water and nitrogen stress identified variables strictly related to plant geometry and leaf area index. Hyperspectrogram manages many hyperspectral images by data extraction and compression while retaining spatial information (Corti et al. 2017). It provides evidence that noninvasive sensors could significantly improve disease detection and quantification in controlled environments by regulating environmental factors to comparatively higher levels. Shortly, horticultural production is expected to have been supported by innovative digital technologies.

1.6 Challenges in Automated Field Sensing

Generally, diseases can easily be detected and quantified through remote sensing if they are widely spread throughout the field and if only the top canopy crop layer is affected by it. Most remote sensing studies have been carried out under controlled conditions using artificial illumination, regulation of incoming light's direction, and sensor placement at a certain angle toward the leaf to absorb desired reflected light. These conditions are not available in the field, so the application of remote sensing technologies for large-scale field assessment is limited.

Among all the factors, light illumination is the most disturbing variable in the field. Leaves in the top most canopy layer appear brighter, in natural illumination source, compared to leaves in lower crop canopy layers. A threshold is required to distinguish between healthy and diseased tissue, which could be achieved by considering the overall image brightness while assessing a specific disease in a particular location. This threshold setting requires intensive research in crop phenotyping. Image brightness shows a higher heterogeneity in the natural canopy when sunny conditions are present, whereas heterogeneities in cloudy weather are less severe because scattered radiation, compared to direct radiation, reaches abundance in the crop canopy. To avoid these radiation variations under field conditions,

modern plant phenotyping setups allow plant disease assessment/monitoring at the appropriate time, such as dawn and dusk, when direct radiation on the crop canopy is limited. Sophisticated machine and deep learning approaches are incorporated into hyperspectral imaging and expert opinion to define, detect, and refine the traits extracted from a hyperspectral image.

1.7 Other Indirect Methods of Plant Disease Detection in Phytopathometry

Thermography differentiates the plant samples based on surface temperature. Thermographic cameras capture the emitted infrared radiations from the plant surface, and then color differences are analyzed. It has been reported that pathogenesis increases the plant surface temperature due to rapid cell activity; hence, thermographic techniques could prove effective in directly detecting plant diseases under field conditions. Thermographs have also been reported to monitor the pathogen heterogeneity in soil-borne diseases. However, thermographic detection is susceptible and is affected by slight environmental conditions (Stoll et al. 2008; Oerke et al. 2011).

Profiling of the volatile organic compounds, released upon plant–pathogen interaction and are highly indicative of stress type, has also proved effective in phytopathometry. Crown rot fungus of strawberry (*Phytophthora cactorum*) causes plants to release characteristic *p*-ethylguaiacol and *p*-ethylphenol volatile compounds. Hence, profiling of volatile compounds could be used to detect the type and nature of disease infection (Fang et al. 2014).

1.8 Conclusion

Phytopathometry is crucial for plant disease management in the era of precision and sustainable agriculture. Phytopathometry is not limited to plant pathology. Various traditional and modern plant disease measurement approaches have been discussed. Current practices of phytopathometry are complex, require expertise to operate, and are also time-consuming due to massive data analysis. Additionally, most of the approaches are not suitable for actual field conditions.

However, advancements and AI and modern nano-fabricated biosensors have huge potential in phytopathometry.

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Visual Estimation: A Classical Approach for Plant Disease Estimation

2

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Abstract

Quantifying plant disease in terms of disease severity, prevalence, incidence, or intensity is very important to make decisions for applying treatments or management practices. Quantifying any plant disease is based on the estimation methods dependent on visual estimation or image analysis of infected portions. Foreseeing the yield losses, tracking and predicting epidemics, evaluating crop germplasm for the source of resistance against a specific disease, and comprehending basic biological processes, such as coevolution, all depend on accurate estimations of disease severity. Inaccurate or vague disease evaluations may cause wrong conclusions from the data, which may cause inappropriate actions to be performed in disease management verdicts. Visual estimation is a highly traditional technique in phyto-pathometry. In the visual estimation of disease, human eye and brain play significant roles in analyzing the degree of disease. It is well established that the number of lesions in proportion to the infected region affects the accuracy and precision of visual estimations; the more lesions there are concerning the infected area, the more overestimation there is. Variability in raters' disease assessment ability, preferences of disease ratings for severity, amount of lesions and their size, color blindness, and time spent on estimation

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are some factors that can induce error in the visual estimation. Different scales often used in measurement science constitute the foundation for visual assessments of disease severity. Several rating scales evaluate a disease's severity using continuous or discrete data. The severity of the disease was determined using nominal, ordinal, and ratio scales. Using suitable scales, teaching, training, and tools (standard area diagrams) have enhanced the precision of visual estimations. This chapter examines recent technological developments that allow for more objective disease assessment (reliability, precision, and accuracy). Over the past 30 years, as software have become advanced and easier to use, visible light photography and digital image analysis have become progressively more popular. Truthfully, several experimentations are utilizing the image analysis technique to provide extremely precise estimations of infection. The most commonly used software are "Assess," "Sigma Pro," "ImageJ," "Adobe Photoshop," and "Scion Image."

Keywords

Visual estimation · Standard area diagrams (SAD) · Digital image analysis · Disease assessment

2.1 Introduction

There are thousands of justifications for why correct and accurate estimation and measurement of plant diseases are required. Quantitative information on plant diseases is essential for farmers and agriculturists to decide. The quantity and quality of plant products are always negatively affected by plant disease epidemics that ultimately impose a severe risk to food safety and security (Oerke 2006; Savary et al. 2012, 2017; Strange and Scott 2005; Madden et al. 2007). Quantitative information regarding plant disease is predominantly significant in such circumstances where the yield loss must be an evident outcome of disease, in plant breeding and genetics for the determination of resistance source in various germplasm (cultivars or varieties), for taking decisions towards disease management strategies such as application of pesticides (fungicides, bactericides, etc.) to manage the plant pathogens, and is also important to understand the epidemiological facts of plant disease and coevolution (Nutter and Gaunt 1996; Berger 1980; Cooke 2006; Gaunt 1995; James 1971; Kranz 1988; Burdon et al. 2006; Nutter et al. 2006).

Plant diseases have always been considered the most significant threat to human survival because these diseases are likely to cause drought and famine; for example, the Irish potato famine (1846–52) imposed a severe human survival threat. The knowledge regarding the disease assessment is necessary to (1) conduct a disease survey, (2) enhance the understanding of disease epidemiology, (3) determine the crop losses, (4) observe the effect of applied amendments (e.g., pesticides, germplasm, etc.), and (5) establish a threshold to make a decision (James 1974; Kranz 1988; Cooke 2006; Madden et al. 2007; Bock et al. 2010b, 2020). Actual and

accurate disease estimation has always been an essential key to the judicious use of pesticides, while the failure in diagnostics of plant infections caused by pathogens (e.g., bacteria, fungi, viruses, etc.) leads to the non-judicious use of chemicals. For that reason, the scientific communities always widely consider plant diseases by focusing on the biological characteristics of diseases. In precision farming strategies, decision-making optimization is carried out by adopting the most advanced technology. Experts usually carry out the preliminary diagnosis of plant diseases, biological reviews, and visual inspection for visual estimation. However, visual inspection is typically an expensive, laborious, and time-consuming methodology. Therefore, it is very important to adopt advanced and intelligent techniques and methods to visually assess the plant disease prevalence and damages (Saleem et al. 2020; Ul Haq and Ijaz 2020; Ul Haq et al. 2020).

Conventionally, as a result of observation with the naked human eye, the plant disease severity is estimated visually by assigning a value in such a way that if a greater value is assigned to any sample, this will explain the greater will be the disease severity. Oppositely, concerning advanced technologies, the stress signals and amount of disease are directly or in-directly measured by a remote sensing technology-based sensor or instrument (Nilsson 1995; Bock et al. 2010b). Hence, the image analysis could process an image captured in a visible spectrum (VIS) (Barbedo 2013, 2016a; Bock and Nutter 2012; Bock et al. 2010b). The image is captured in a non-VIS spectral range, and chlorophyll fluorescence or other methods can be used to measure the level of disease. Non-VIS spectral range includes multispectral imaging (MSI) and hyperspectral imaging (HSI and MSI). The latter approaches are theoretically different from estimating or measuring the severity of disease solely in the light of visible symptoms and VIS spectrum (Kuska and Mahlein 2018; Mahlein et al. 2012, 2018; Simko et al. 2017; Mutka and Bart 2015). Generally, the wavelength ranges for VIS spectral are 380 to 750 nm, while non-VIS spectral (MSI and HSI) use wavelengths from 250 to 2500 nm. Any single system does not cover this wide range; therefore, NIR (near-infrared) and IR (infrared) are usually chosen as part of the described range.

To estimate the percent diseased portion, the raters observe and learn to differentiate symptomatic tissues from asymptomatic ones. Analysis of images captured in the VIS spectrum is usually based on the measurement of predefined diseased state pixels vs. healthy state pixels recognized by following a range of statistical processes. In hyperspectral and multispectral imaging systems, those specified wavelengths that are linked with the infected or unhealthy state are measured. However, some additional challenges can be faced in image acquisition and analysis, but this technique has several advantages over the visual estimation of plant diseases (Mahlein 2016). Contingent upon the objective, just like visual assessments and ratings of plant diseases, image-based analysis systems should be able to: (1) identify the infection or any other disorders as quickly as feasible, (2) distinguish amongst biotic maladies, (3) discriminate between biotic maladies and abiotic stresses, and (4) measure the disease severity very precisely (Bock et al. 2020).

2.2 Significance of Precise Quantification of Plant Disease Severity

A precise estimation or quantification of symptomatic plant tissues based on visual assessment is similar to the definite or true value (Bock et al. 2010b, 2016). For remote sensing techniques, the precise/actual reference values are mentioned as "ground truth" data. Those measurements or assessment data obtained from visual estimation that differs from actual reference values are known as "biased" data. Generally, two types of biases occur (a) systematic bias and (b) constant bias. Precision is the inconsistency of assessments; however, for the estimation or measurement accuracy of plant disease severity, the precision must be very close to the actual value (Madden et al. 2007). According to the definition, continuously precise assessments should be reliable, where consistency is the propensity for recurring assessments or measures of the similar sample(s) close to each other (Bock et al. 2016; Madden et al. 2007; Nutter et al. 1991).

Quantification or estimation of disease severity is of great importance. It should be very precise, as an accurate estimation ensures that (a) the effect of applied treatments is appropriately evaluated, (b) the association of losses in production is understood, (c) the conducted surveys are eloquent, and (d) the germplasm phenotypes evaluated correctly. Additionally, the disease severity data collected from visual estimation can also be used to decide the economic threshold level or for disease forecast that may help determine the application of treatments. Inaccuracy in disease estimation can lead to severe consequences such as obstruction in the research procedures, wastage of time, and precious resources, and in the end, it can deleteriously affect the farmers' profit. The requisite degree of accuracy and precision in the estimation process can be varied from situation to situation. Numerous experimental and simulation studies have shown that, as a result of estimating the disease, the type II error may occur as a result of incorrect disease assessment (a false negative, which indicated a failure in null hypothesis rejection) (Chiang et al. 2014, 2017a, b; Christ 1991; Newton and Hackett 1994; Bock et al. 2010a; Parker et al. 1995a). The type II error will seriously affect the decisions taken from those experiments. Decisions based on such conclusions could cause wastage of resources, expansion in infection, losses in production, and eventually reduced revenue. Therefore, the precise estimation of plant disease would seem very important (Bock et al. 2021a). This type II error can be minimized by accurately estimating and quantifying.

2.3 Terms and Definitions Regarding Disease Estimation and Quantification

Those terminologies are used to narrate the notions, and their interpretation is critical in the research area of plant disease estimation. Because of the advancements made in the related fields, including measurement science, these terminologies must be redefined (Madden et al. 2007). In 1953 and 1966, Large launched the term

"Phyto-pathometry" or "plant pathometry." These terminologies are considered a branch of plant pathology that estimates plant disease (Large 1953, 1966). The term phytopathometry was derived from three Greek letters (a) phyto means plant, (b) pathos means disease, and (c) metron means to measure. The term phytopathometry was also employed by Nutter et al. (2006) and Horsfall and Cowling (1978). Based on the description provided by Large (1953, 1966), the term phytopathometry deals with the assessments, guiding principles, and history of plant disease symptoms. Moore (1949) was the first scientist who made some initial efforts to discuss the significance of plant disease estimation; however, Chester (1950) accomplished the first conclusive literature review. The necessity of comprehensive terminologies in phytopathometry was admitted by the 1980s when a subcommittee was founded under the American Phytopathological Society (APS) that provided unanimity on the terms and concepts of the disease estimation (Nutter et al. 1991).

Various methods for estimating plant disease symptoms quantify the disease's prevalence, severity, intensity, or incidence. Commonly, two terms are used to describe the plant disease data, i.e., "estimate" (adopted to describe the results of assessments made on visual observation) and "measurement" (used for the assessments performed using hyperspectral or image analysis technology). Disease prevalence is the ratio or percentage of fields, states, countries, continents, etc. where the disease has been perceived. Disease incidence is the ratio or percentage of the diseased plant (or plant units, leaves, branches, fruits, etc.) out of the total number of assessed experimental units (Madden et al. 2007). Disease incidence equates to disease prevalence, but disease prevalence is used to describe the disease at a grander scale than incidence. Disease severity is the proportional description of the sampling unit's relative or absolute diseased area (fruits, stem portion, leaf, branches, etc.). Disease severity can also be described as the degree to which the sampling unit (plant, stem portion, fruits, branches, leaf, etc., or plot) is infected (Nutter et al. 1991). Disease intensity is a usual and broad-spectrum terminology used to narrate the quantity of disease detected in a described or defined populace (Nutter et al. 1991; Bock et al. 2021b).

2.4 Visual Assessment of Plant Diseases

With the passage of time and advancements made in the field of disease estimation, various reviewers and plant pathologists have documented the developing prominence of visual assessment. Some of the most important literature on plant disease visual assessment revealed that visual estimation is a classical approach in phytopathometry. Throughout the course of about last 140 years, starting from the principal tool was made as a guide for the quantification of disease severity (Cobb 1892), different perspectives have been established to improve and standardize the precision of visual evaluations of plant disease severity (Anon 1947; Madden et al. 2007; Bock et al. 2010b, 2016, 2021b; Campbell and Madden 1990; Horsfall and Cowling 1978; Chaube and Singh 1991b; Chester 1950; Chiang et al. 2014; Cooke

2006; James 1974; Kranz 1988; Large 1966; Nilsson 1995; Nutter 1999; Del Ponte et al. 2017; Nutter and Esker 2006). After 2010, several reviews described the importance of visual estimation in phytopathometry.

2.4.1 Role of Human Eye and Brain in the Visual Estimation of Plant Disease

The most primitive remote sensing device is the human eye which works in combination with the human brain, where the human brain performs a system similar to image analysis (Nilsson 1995; De Jong and Van der Meer 2007). The functionality of the human eye for creating a picture and the principle of the camera to operate are much more similar. The part of the eye that receives and focuses a wide range of light rays is called the cornea, which bends these rays through the pupil. The iris surrounds this part of the eye (pupil). The function that is performed by the iris and pupil in the eye is done by the aperture in the camera. Then the inbound light inside the eye comes upon the lens that aids the light focus on the part of the eye at the back called the retina. This part of the eye is surrounded by photoreceptor nerve cells. The light rays are converted by these photoreceptor nerve cells into electrical impulses and then the optic nerves transmit these electrical impulses to the human brain, where an image is created as the result of the translation of these rays. In visual estimation, it is noteworthy that the eye and all the relevant brain tissues involved in acquiring the image, analyzing, and interpreting an object work very fast (Hubel 1995). With the help of the brain and eye functioning, the observer can estimate the effects and extent of the disease on any particular plant, branch, or leaf. In the previous 30 years, to our understanding, significant progress has been made in the factors that play an important role in the quality of visual estimation of diseases and their measurement, and this information about the progress has been utilized to develop such methods that can help in improving the rater's ability (Horsfall and Cowling 1978; Berger 1980; Chester 1950; Horsfall 1945; James 1971; Large 1966; Nutter and Esker 2006).

2.4.2 Factors Inducing Error in the Visual Assessment of Diseases in Plants

The process of estimating the visual disease severity is likely to face several errors. This has been acknowledged since the beginning of phytopathology; however, the knowledge of these sources of inaccuracy has only lately been grounded in empirical research. Many factors and causes regarding errors induced in the rater's estimation, in different pathosystems, have been recognized and measured. Indeed, Smith et al. (1969) were the first researchers who quantified the error found in visual estimation of tomato leaf mold disease caused by Cladosporium.

2.4.2.1 Variability in Raters' Disease Assessment Ability

Individual ability varies, as shown by several pieces of research using various pathosystems. Differences amongst individual and individual variations between disease assessments are highlighted by inter-rater and intra-rater reliability and measures of agreement (Amanat 1976, 1977; Newton and Hackett 1994; Bock et al. 2008a, 2009c; Guan and Nutter 2003; Nutter et al. 1993; Sherwood et al. 1983). Nutter et al. (1993) were the first to establish rater variability. However, Sherwood et al. (1983) revealed the effects of rater capability in their research, differentiating the rater estimations of infection induced by a fungus (*Stagonospora arenaria*) on the leaves of the *Dactylis glomerata* plant. The rater variability was also studied by Bock et al. (2009a). Some people are innate, to be precise, whereas others do not. Individual raters have a propensity to overestimate or underestimate; this propensity may be constant over the entire disease rating scale; alternatively, the rater might have to change propensities across the percentage scale's range (Bardsley and Ngugi 2013; Bock et al. 2009b; Godoy et al. 2006; Hau et al. 1989; Nita et al. 2003; Schwanck and Del Ponte 2014; Yadav et al. 2013). Regarding rater bias, quantitative ordinal scales can exacerbate type II error (Chiang et al. 2016a).

2.4.2.2 Preferences of Disease Ratings for Severity

Various evidence concerning the fact that the raters mostly choose "preferred values" as a result, but more research is needed to understand this tendency fully. Individuals tended to favor specific values when estimating disease, according to Hau et al. (1989) and Koch and Hau (1980), who found that the individuals picked these values more frequently than predicted if the assessment was randomized based on the original conditions. Raters have presented a steady inclination for definite severities at intervals of 5%, and mostly 10% for the severities counted as greater than 10%. As a consequence, raters favor the intervals of 10%, 15%, 20%, 25%, . . ., 95%, and 100%, this methodology used to account for the severities can bring about possible errors (Bock et al. 2008a; Koch and Hau 1980; Schwanck and Del Ponte 2014).

2.4.2.3 Amount of Lesions and Their Size Give an Overestimation of Disease Severity

At low disease severity levels, there is a typical tendency to overestimate, which is especially sensitive to the quantity and lesion size; more number of lesions present, the greater the trend to exaggerate or overestimate (Bock et al. 2008a; Nita et al. 2003; Sherwood et al. 1983; Forbes and Jeger 1987). Amanat (1976) discovered that regions beneath extensive lesions had lower mistake rates than minor lesions in the same area; similar results were also described by Hau et al. (1989). When compared to minor, random, or uniformly distributed lesions, it was also discovered by Forbes and Jeger (1987) that estimating the disease severity owing to fewer bigger lesions was less prone to error. Overestimation was reported to be widespread among raters by Godoy et al. (2006). Although it is widely known, the source of overestimation does not appear to have been adequately investigated (Amanat 1976; Bock et al. 2008b, 2009a; Beresford and Royle 1991; Sherwood et al. 1983).

2.4.2.4 Structural Characteristics of Host and its Size

The quality of the severity assessment might be influenced by the plant structure being evaluated. It is worth noting, though, as a possible cause of error among units (Shokes et al. 1987; Christ 1991; Danielsen and Munk 2004; Forbes and Jeger 1987; Townsend 1943; Vereijssen et al. 2003). Based on compared estimations, it was discovered by Forbes and Jeger (1987) that plant structure had a substantial influence on disease, and they also revealed that the mean absolute error was between the range of 0.30% and 15.90% with mean absolute error ranging from 0.3% to 15.9% across the various plant architectures tested. Moreover, it demonstrates the significance of organ evaluation in connection to infection severity. The impact of the size of plant leaf was observed by Nita et al. (2003) and they discovered a tiny but substantial influence of the size of the leaflet on Phomopsis on strawberry severity assessment. Even though information regarding the creation and authentication of standard area diagrams may be helpful in this field, most diagrams are created for foliar diseases (Del Ponte et al. 2017).

2.4.2.5 Time Spent on Estimating Plant Disease

It was found by Parker et al. (1995b) that accuracy was impacted by the time it took the evaluators to grade the disease; while quick evaluations weren't always less accurate, they were typically less accurate than delayed assessments. There is limited information on how long it takes to assess the severity of a condition, but on average, direct visual assessments of severity take around 7 s (Bock et al. 2009a; Martin and Rybicki 1998). Nutter et al. (1993) described that almost 32 min are required for the rater to evaluate the disease severity of Stagonospora on a defined quadrat with the dimension of 80×1 m^2 of bentgrass.

2.4.2.6 Color Blindness

Persons with color blindness have a reduced capability to analyze the disease. The intensity of red/green colorblindness affects the estimate of infection severity on golf greens (Nilsson 1995).

2.4.2.7 Interactions Between Multiple Factors

Several pieces of research have found interactions between numerous components although they have seldom been thoroughly investigated. In an analysis of variance involving a variety of factors, it was found by Bock et al. (2008b) and Sherwood et al. (1983) that there are some interactions between multiple factors such as actual area, individual rater, number of lesions, and rater groups. Nita et al. (2003) discovered numerous variables impacting disease estimations, including real infection, number of lesions, and the size of leaflets, but the latter had minimal effects. Finally, sampling strategy and the sample size must be considered to produce an impartial assessment of disease severity (Steddom et al. 2005; Madden et al. 2007).

2.4.3 Methods for the Visual Assessment of Infection Severity in Plants

In 1917, the APS organized a committee that convened for several years (Anon 1917). A few years later, in 1933, the BMS formed a related committee to examine methods of recording and measuring disease occurrence and intensity (Large 1955; Moore 1943; Beaumont et al. 1933). Until 1950, the progress of disease estimation techniques was summarized by Chester (1950), as well as the expertise and technique advancements related to the young field of plant disease severity evaluation. A review of the many approaches used to measure disease severity visually is necessary, both historically and to assess their usefulness, applicability, and scientific basis. The visual evaluation of infection severity is based on many scales used in measurement science (Stevens 1946; Baird and Noma 1978). Several rating systems evaluate the severity of diseases using continuous or discrete factors (Sheskin 1997). Some attempt to be general, while other rating scales are personalized to specific phytopathogenic systems (Campbell and Madden 1990; Chester 1950; Madden et al. 2007; Nutter and Esker 2006). Because they lack a true zero, only interval scales are not taken into account for estimating the severity of plant diseases (it is impossible to estimate less than zero diseases). Nominal, ordinal, and ratio scales were used to assess the disease's severity. The following scales are used in visual plant disease evaluation:

2.4.3.1 Nominal or Descriptive Scales

These are the most fundamental and perhaps most arbitrary metrics for determining the severity of infection (Nutter and Esker 2006; Chester 1950). These descriptive (qualitative) disease rating scales have previously been described and characterized in the literature (Bock et al. 2010b, 2016; Campbell and Madden 1990; Madden et al. 2007; Newell and Tysdal 1945). The disease is classified into several categories. Nominal scales are based on short descriptors that define the degree of disease, such as "no," "minor," "moderate," and "severe," or symbols such as "−" for no disease, "+," "++," and "+++" for the different magnitude of infection severity. Nominal scales are arbitrary and might vary based on the rater and the evaluation period. The data may be evaluated using statistical techniques based on rank or frequencies.

2.4.3.2 Ordinal Scales

Plant pathologists frequently face circumstances where measuring nearest percent estimates (NPEs) of disease severity is either time demanding or quite impracticable. In these circumstances, the examiners often adopt an ordinal (quantitative or qualitative) scale for disease assessment (Shah and Madden 2004; Bock et al. 2010b). An ordinal scale illustrates the order of disease estimation or their ranking; however, the variations between the assumed classes are typically neither equivalent nor equal and may be quantitative, qualitative, or a combination of the two. These have been termed ordinal scales, interval scales, quantitative ordinal scales, and category scales in the literature (Bock et al. 2009c, 2016; Nutter and Esker 2006; Chiang et al. 2014; Hartung and Piepho 2007). The common alternative of nearest percent estimates

Table 2.1 A standard scale with 0–5 values was commonly used for severity assessment of watermelon mosaic virus and zucchini yellow mosaic virus on watermelon (after Xu et al. 2004)

Scale	Severity of disease symptoms
0	No visible symptoms
1	Slightly mosaic symptoms on leaves
2	Mosaic patches and/or necrotic lesions on leaves
3	Leaves near apical meristem deformed slightly, yellow in color, and reduced in size
4	Apical meristem with mosaic symptoms and deformation
5	Extensive mosaic and severe deformation of leaves (or plant dead)

(NPEs) is using ordinal scales to assess the disease severity. The severity of any plant disease acquired with an ordinal rating scale is a numeric variable although such kind of disease rating scale is based on symptoms' descriptions (Madden et al. 2007; Agresti 2007, 2010; Larrabee et al. 2014). The order of values in the qualitative ordinal scale is reasonably clear and significant; however, the numeric value of the differences is mostly unknown amongst each class. The "Likert scale" is an example of a qualitative ordinal scale (Likert 1932). The qualitative ordinal scale was developed based on some subjective classes of 1 = very little disease, 5 = medium disease, and 9 = very much disease. Such very concise and simple ordinal disease scales are very comprehensive and cannot be exchanged between location, raters, and situation (Chester 1950). By assigning a number to a symptom explanation, the disease's stage of development may be quantitatively assessed or signified. Such as watermelon diseases, i.e., watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV), have been evaluated on a six-classed (0–5) scale presented in Table 2.1 (Xu et al. 2004).

Conversely, the other type of ordinal scale is the quantitative ordinal scale which describes the successive numerical intervals or ranges. Such ordinal scales are usually based on disease symptoms with the percentage areas with specified differences between each class/category. The order of values for these ordinal scales is clear and significant, and each ordered number's magnitude is numerically constrained by a specific range (Bock et al. 2020). One of the important examples of this quantitative ordinal scale is the Horsfall–Barratt (H-B) disease rating scale. According to this scale, the disease severity from 0% to 100% was divided into 12 consecutive intervals based on logarithm, as shown in Table 2.2 (Horsfall and Barratt 1945). Numerous quantitative ordinal scales that divide the percent scale into different classes and interval sizes have been devised (Hunter and Roberts 1978; Hunter 1983; Forbes and Korva 1994; Hartung and Piepho 2007; Bardsley and Ngugi 2013; Chiang et al. 2014). Rating scales, commonly used for the estimation of diseases such as root diseases, systemic diseases, and diseases caused by several viruses, may be included in the category of quantitative ordinal scales, such as rating scales for diseases caused by cassava mosaic virus (CMV) and citrus greening disease that is also known as huanglongbing of citrus (Hahn et al. 1980; Gottwald et al. 2007). Another example in the support of this fact is Chiang et al. (2014), who showed that a 10% linear scale emphasizing severities ≤50% infection and having

Table 2.2 The quantitative ordinal scale proposed by Horsfall and Barratt (H-B) shows the severity of disease and interval size ranges with their midpoints (Horsfall and Barratt 1945)

H-B categories	Midpoint	Ranges for infection severity	Disease-free ranges	Interval size
1	0	0	100	0
2	1.5	0^+ to 3	97 to <100	3
3	4.5	3^+ to 6	94 to <97	3
4	9.0	6^+ to 12	88 to <94	6
5	18.5	12^+ to 25	75 to <88	13
6	37.5	25^+ to 50	50 to <75	25
7	62.5	50^+ to 75	25 to <50	25
8	81.5	75^+ to 88	12 to <25	13
9	91.0	88^+ to 94	6 to <12	6
10	95.5	94^+ to 97	3 to <6	3
11	98.5	97^+ to 100	0 to <3	3
12	100	100	0	0

some extra categories at low disease severities, i.e., ≤10% may be a viable alternative for estimating the disease severity (%) when a quantitative ordinal scale is selected preferably. In the section of instructions to authors, the APS instructs them to use an ordinal scale (APS 2020).

Equal or unequal intervals can be found among the categories or classes of quantitative ordinal scales (Horsfall and Barratt 1945; Horsfall and Heuberger 1942; Hunter and Roberts 1978). Horsfall–Barratt (H-B) scale is the most extensively used disease rating scale (Haynes et al. 2002; Jones and Stansly 2014; Kutcher et al. 2018; Miyasaka et al. 2012; Rioux et al. 2017; Strayer-Scherer et al. 2018). H-B scale is, however, established on the nonexistent Weber–Fechner law (that is related to human perception) (Nutter and Esker 2006), and raters' ability to assess in extensive categories in the middle of the scale is superior than what the scale suggested (Bock et al. 2009b; Nutter and Esker 2006; Forbes and Korva 1994). The findings of plant breeding experiments show an inappropriate scale structure (Xie et al. 2012). An amended quantitative ordinal scale has been established that offers a lesser possibility of type II error; this amended quantitative ordinal scale is endorsed wherever an ordinal scale is necessary (Chiang et al. 2014) (Table 2.3). Similar to the Cobb disease rating scale (Cobb 1892), other disease estimation interval scales are frequently supplemented by standard area diagrams (Large 1966; Chester 1950). The opinion reinforced the establishment of these ordinal scales that descriptive scales were less-than-ideal; certainly, Horsfall and Heuberger (1942) and McKinney (1923) established early interval scales to measure infection severity in a more quantifiable manner (Table 2.4).

An ordinal score's frequency can be adopted to calculate the disease severity index (DSI%) (Chester 1950). A rater uses the scale to quantify the severity of the disease on the specimens, and the result is used to calculate the disease severity index

Table 2.3 An improved quantitative ordinal scale with 16-class values for the general assessment of plant disease severity based on the scale developed by Chiang et al. (2014)

Ordinal equivalent	Disease severity range (%)	Midpoint
0	–	–
1	0^+ to 0.1	0.05
2	0.1^+ to 0.5	0.30
3	0.5^+ to 1.0	0.75
4	1.0^+ to 2.0	1.50
5	2.0^+ to 5.0	3.50
6	5.0^+ to 10.0	7.50
7	10.0^+ to 20.0	15.0
8	20.0^+ to 30.0	25.0
9	30.0^+ to 40.0	35.0
10	40.0^+ to 50.0	45.0
11	50.0^+ to 60.0	55.0
12	60.0^+ to 70.0	65.0
13	70.0^+ to 80.0	75.0
14	80.0^+ to 90.0	85.0
15	90.0^+ to 100.0	95.0

Table 2.4 An early interval scales proposed by Horsfall and Heuberger (1942) for disease severity assessment in a more quantitative way

Category	Disease severity in terms of leaf area
0	Superficially no sign of infection
1	From trace to 25% infected area of leaf
2	From 26% to 50% infected area of leaf
3	From 51% to 75% infected area of leaf
4	More than 75% infected area of leaf

(%) (Chaube and Singh 1991a; Chester 1950; Vieira et al. 2012; Hunter and Roberts 1978; Kora et al. 2005).

$$\text{DSI}\ (\%) = \frac{[\sum(\text{score of rating class} \times \text{class frequency})]}{[(\text{maximal disease index}) \times (\text{total number of plants})]} \times 100$$

Although there may be a correlation between real severity and a severity index, the two terms cannot be used synonymously because of their fundamental differences. If the rating class's midpoint values are ignored, recent investigations by Chiang et al. (2017a, b) showed that the Disease Severity Index could be principally vulnerable to overestimation when employing the aforementioned approach.

2.4.3.3 Ratio Scales

Such types of scales are percentage-based scales that are commonly extensively utilized in assessing plant diseases in several pathosystems to determine the severity of any plant disease. To the best of the rater's capability, the continuous percent scale has the following benefits: (a) the limits (upper and lower) of a ratio scale are constantly defined, i.e., 0.0% and 100.0%, (b) this scale is widely recognized,

(c) divisions and sub-divisions are easy to make, and (d) it is commonly used as a method of measuring area coverage. Regarding evaluation accuracy and dependability, raters differ significantly (Bock et al. 2010b).

Numerous diseases allow for the estimate of severity using ratio scales. A rater measures the percentage of the plant parts exhibiting disease symptoms and determines the severity using a percentage scale that spans from 0% to 100%. Many people use the % scale to visually judge severity (latest cases include Bock and Chiang 2019; Gent et al. 2018; Xue et al. 2019; Hamada et al. 2019). The data on the % scale may be analyzed using some suitable statistical tools like parametric statistics and standard deviations.

Few researchers have examined how to decrease type II error chances while minimizing the utilization of specimen numbers and evaluation techniques in visual disease assessment (Chiang et al. 2016b). The study's findings showed that significant factors must be under consideration for enhancing the validity of hypothesis tests include the selection of assessment techniques, optimizing specimen counts, and the number of duplicate estimations while adopting a balanced experimental design (Bock et al. 2020).

2.4.4 Strategies to Enhance the Accuracy of Visual Estimates

A careful comparison between estimated values and actual values must be considered to detect and quantify error features. Once the inaccuracy has been established, methods for correcting it may be created, and subsequent improvements can be assessed against the original data. As a result, creating a baseline of understanding mistakes is critical throughout the process to identify the origins and assess improvement. Several investigations conducted over the last 30 years have proven the range of skills among both experienced professionals and inexperienced nonprofessionals visual assessors in a variety of pathosystems with a wide range of infection symptoms (Amanat 1976; Bock et al. 2008a, 2009c, 2010b; Kranz 1988; Nita et al. 2003; Nutter et al. 1993; O'Brien and van Bruggen 1992; Sherwood et al. 1983; Weber and Jorg 1991). The approaches listed below can be used to increase the accuracy of estimates:

2.4.4.1 Computer-Based Training

It was exposed by Nutter and Schultz (1995) that the computer-based trainings play an important role to enhance precision levels; however, this effect may be transient (Parker et al. 1995b). On the other hand, training may diminish accuracy in a few circumstances, presumably due to the training on some specific pathosystems which are unrelated to the one utilized in practice (Bardsley and Ngugi 2013). It was also discovered by Nutter and Schultz (1995) that the coefficient of determination (R^2) of a rater was increased from 0.825 to 0.933 after the completion of computer-based training. The coefficient of determination (R^2) describes the precision level. Training software applications such as DISTRAIN (Tomerlin and Howell 1988) and severity were created for earlier computer operating systems (Nutter and Litwiller 1998).

There are no new or updated versions of these computer-generated image-based training programs; they may have been superseded by training raters using true-color photographs of symptoms paired with standard area diagram technology.

2.4.4.2 General Field/Lab Training

The capacity to estimate properly is influenced by experience in detecting infection signs. Although some novice raters may be more accurate than others, skilled raters tend to be more precise as a group (González-Domínguez et al. 2014; Yadav et al. 2013). Training raters improve reliability and agreement, historically done with field material (Nutter and Schultz 1995). The disadvantages are that it is frequently a busy time of year and that training needs field trips. As samples age, they lose disease-specific characteristics. Training raters in the field is undoubtedly valuable, but this has not been fully explored, most likely due to logistical constraints and the simplicity of training provided by SADs and computer assessment programs. Although not in the field, Amanat (1976) demonstrated that repeated training with feedback improved the precision of disease assessment by numerous raters by using photographs of leaves with varying-sized lesions and areas affected.

2.4.4.3 Role of Standard Area Diagrams (SADs) Towards Improved Accuracy

A straightforward and extensively adopted method for improving the accuracy and precision of rater estimations is the development of standard area diagrams. Cobb's (1892) diagrams are the oldest evaluation aid. SADs were later created by James (1971) for various crops. Over the previous 2.5 decades, investigation on standard area diagram creation and authentication has increased, which highlights the significance of SADs in enhancing precision. Gains from SADs vary across raters and throughout pathosystems (Schwanck and Del Ponte 2014; Spolti et al. 2011) and are often higher for the least accurate raters (Braido et al. 2014; Debona et al. 2015; Duan et al. 2015; González-Domínguez et al. 2014; Yadav et al. 2013). Vary from >0.4 for novice raters to 0, with inherently accurate raters seeing a little loss in agreement. Overall, using standard area diagrams helps standardize raters, improving inter-rater reliability (a result of the accuracy of severity estimates on individual specimens) (Domiciano et al. 2014; Duarte et al. 2013; González-Domínguez et al. 2014; Spolti et al. 2011). Although some are more cautious, this is an excellent agreement in measurement science (Altman 1990). When standard area diagrams are not employed, the agreement is frequently less than 0.85. There may be symptomatic patterns in which unassisted assessments are highly correct, so SADs are less valuable (Bock et al. 2020).

2.4.5 Implementation in Experimentation and Its Future

Visual evaluations are the most commonly used at the level of distinct organs, plants, and, sometimes, at the field level. The statistics obtained are, however, employed at the local and universal levels. Estimating disease severity visually at the field level is

legitimately outdated. Evaluation of disease severity has been used to calculate the consequence of applied treatments, measure the influence of disease on production, conduct surveys, and estimate the disease severity on various varieties/ cultivars, among other things. For instance, in the 1950s, a key (rating scale) was devised to assess the late blight of potatoes at the field level in the United Kingdom (Moore 1943). Although such kinds of field estimation keys are acceptable for disease severity evaluation, they are not studied further because they have not been used very commonly in recent years.

Visual grading systems will be the most important method of evaluating plant diseases in the predictable future. Undoubtedly, newly developed technology will continue to provide more. Therefore, the best techniques for assessing disease severity, determining the degree of estimation error, and minimizing that error is highly sought. Consider the accuracy and agreement required for the disease assessment task while selecting an assessment system. Studies should, if feasible, employ approaches that maximize rater consistency and agreement with the authentic values.

2.5 Estimation of Plant Diseases by Adopting Image Analysis Technique

Digital cameras are a low-cost and readily available resource that may be utilized for various plant pathology applications. Photography (digital and formerly film) has been used for many decades to identify, measure, and investigate diseases and infections. In the 1920s, aerial film photography of infected agricultural fields was started (Neblette 1927), which helped the farmers and researchers in the detection of disease and its quantification (Johnson et al. 2003; Bauer et al. 1971; Toler et al. 1981; Brenchley 1964; Colwell 1956; Jones et al. 2006; Manzer and Cooper 1967; Schneider and Safir 1975; Wallen and Jackson 1971). In the plant pathology, digital image analysis has been used to measure and analyze microscopic pathogen and host physiology and development (Diéguez-Uribeondo et al. 2003; Hilber and Schiiepp 1992; Seiffert and Schweizer 2005; Smith and Dickson 1991) along with dispersal of the pathogen (Diéguez-Uribeondo et al. 2003; Fitt et al. 1982; Hilber and Schiiepp 1992; Smith and Dickson 1991;).

The assessments made based on image analysis under the visible spectrum have the potential to be precise, repeatable, and replicable (Barbedo 2014; Bock et al. 2008b; Clement et al. 2015; Martin and Rybicki 1998). Lindow and Webb (1983) were among the early innovators of plant disease and digital image analysis. More sophisticated algorithms and statistical techniques, particularly after 2000, have increased the ability to distinguish symptomatic diseased tissue from healthy tissues in digital pictures (Bock and Nutter 2012; Barbedo 2013, 2016a, 2017, 2019).

2.5.1 Role of Digital Equipment Toward Image Acquisition

The most common equipment used for image acquisition is slide scanners, video cameras, traditional film cameras, flat-bed scanners, and digital cameras. Digital cameras have quickly replaced film cameras as the principal means of imaging samples. There is a wide variety of capacities, and it would be unreasonable to try a summary of their advantages and disadvantages. This section will offer a high-level review of digital images related to picture capture. The latest research was published in an American journal named plant disease. The study explained the information regarding digital images and digital camera-based image capturing techniques (Ricker 2004); this published material explained some elements of this useful technology to phytopathologists. A flat-bed scanner can also be used to produce digital pictures. This equipment is used to process negatives of photographs and old photographic prints. Information is then received and flowed through the imaging process, from the choice of the sample unit to record to the measurement of the sick region (Price and Osborne 1990; Nilsson 1995).

Different cameras and image capture systems record in the visible spectrum. Portable red-green-blue (RGB) sensors are commonly accessible. With the introduction of portable cameras, the possibility of conveniently collecting countless photos has multiplied (Pethybridge and Nelson 2015). The use of digital video sensors, analog video cameras, and flat-bed scanners for the acquisition of images is very common (Clement et al. 2015; Hetzroni et al. 1994; Kwack et al. 2005; Lindow and Webb 1983; Berner and Paxson 2003; Lloret et al. 2011; Olmstead et al. 2001; O'Neal et al. 2002; Škaloudová et al. 2006; Martin and Rybicki 1998).

2.5.2 Protocols for Analysis of Images and Their Processing

2.5.2.1 Common Software Used for Image Analysis

The researchers, during their research work, use some specific software provided by third-party for the assessment of disease severity in plants, such as a software named "Assess" (De Coninck et al. 2012; Bock et al. 2008a, b, 2009a, b, c; El Jarroudi et al. 2015; Horvath and Vargas 2005; Sun et al. 2014; Mirik et al. 2006; Steddom et al. 2005), which was originally introduced during the year of 2002 (Lamari 2002). While using the software "Assess," the users have to define the segmentation parameters for automation before proceeding; however, this only works if all photographs were collected in similar environments (Bock et al. 2009a). Sigma Pro, ImageJ, Adobe Photoshop, and Scion Image Software are examples of other software (Peressotti et al. 2011; Berner and Paxson 2003; Abràmoff et al. 2004; Kwack et al. 2005; Olmstead et al. 2001; Laflamme et al. 2016; O'Neal et al. 2002; Stewart and McDonald 2014; Cui et al. 2010; Wijekoon et al. 2008; Goodwin and Hsiang 2010; Kerguelen and Hoddle 1999). There were 20 programs described in the SAD evaluation to acquire real severity measures although Assess and Quant were the most widely utilized (Vale et al. 2003; Del Ponte et al. 2017).

2.5.2.2 Processing of the Image

After obtaining the image, it may be altered using various image processing and analysis software programs. Color and contrast may be adjusted; photos can be rotated, sharpened, reversed, or modified. Adobe Photoshop (Adobe Systems Inc., San Jose, CA) is a comprehensive software suite that provides several choices for picture enhancement. Most image analysis systems provide editing and alteration tools, such as edge enhancement, geometric adjustments, etc.

2.5.2.3 Validation of Assessment

The image assessed measurement is validated by comparing it to an authentic or "gold standard" reference. A visual assessment may determine the real value (El Jarroudi et al. 2015; De Coninck et al. 2012; Steddom et al. 2005) or manually determined image analysis data (Bock et al. 2009c; Martin and Rybicki 1998; Peressotti et al. 2011). Although for assessing the accuracy of the image analysis system, the regression analysis has regularly been incorporated in the studies (Horvath and Vargas 2005), however, additional statistical principles are frequently utilized to give further significant understandings (Stewart and McDonald 2014; De Coninck et al. 2012; Bock et al. 2009c). As the investigational systems and circumstances differ among investigations, therefore the findings are not all the time analogous; stated variances based on regressions/coefficient of determination (R^2) and correlations coefficient (r) lie between the range of 0.70 and 1.00 (Martin and Rybicki 1998; Peressotti et al. 2011; Steddom et al. 2005; De Coninck et al. 2012).

2.5.3 The Preciseness of Image Analysis

In recent years, there has been an increase in the number of researches using Convolutional Neural Network (CNN) (a technique used for entity identification and classification under deep learning). To automatically recognize two severity categories of cassava mosaic virus disease (CMVD), Ramcharan et al. (2019) utilized a convolutional neural network and 2415 leaf samples from the cassava plant. The precision for the detection of low severity was observed as 29.40%. According to Esgario et al. (2020), the accuracy of deep learning was up to 84.13% when determining the severity of various coffee infections. According to the research of Wang et al. (2017), the disease severity percentage of black rot disease of apple leaf, based on the four severity categories, varied between 83.3% and 100%. Therefore, compared to visual assessment utilizing a 0–100% scale, accuracy estimations are frequently considered at a lower resolution. For a hypothesis test, the scale type, the number of intervals in the rating scale, and the replications may differ significantly (Bock et al. 2010a; Chiang et al. 2014, 2016a, b, 2020).

Low accuracy is frequently reported in studies employing VIS spectrum photos taken in the field. Two factors are likely to be the leading causes of the variance in image analysis. First, the circumstances in which the photos were taken and the symptoms' range. Examples of systems suggested by Hu et al. (2017), Barbedo

(2017), and Macedo-Cruz et al. (2011), which produced images with 84%, 91%, and 92% precision, respectively, are examples of images taken in variable conditions; while the systems recommended by Stewart et al. (2016), Kruse et al. (2014) and Patil and Bodhe (2011) are the examples of images taken in controlled environments that exhibited in 94%, 95%, and 98% precision, respectively. Second, accuracy will be impacted by the exact reference values used to compare the estimations. Subjectivity will be closely tied to the rater's perceptions when the reference is a visual estimation (Bock et al. 2008b).

2.5.4 Error Sources Impacting Accuracy

2.5.4.1 Machinist

The system operators must correctly match the symptoms to the diagnosis criteria. However, measurements made by hand utilizing image analysis are vulnerable to error. Actual results from image analysis that are used to verify automated methodologies (or other evaluation techniques) are uncertain (Bock et al. 2008b; Barbedo 2013). But the error should be small.

2.5.4.2 Differences in Infection Signs, Host, and Background

For deep learning models to be effective, they must be trained on the images that cover a variety of scenarios. For most alternative procedures, segmenting the leaf and the disease is necessary (Barbedo 2016a). The variations in weather conditions, reflections, contrasts, brightness, and several many other variables are of much importance for developing the threshold values. The variation in these aforementioned factors affects the threshold values that are generally developed for one set of circumstances and when these factors variate the threshold values also become changed for a new set of circumstances (Barbedo 2014). Relying on the circumstances such as how environmental variables interact, and the growth stage, symptoms may change (Patil and Bodhe 2011; Mutka et al. 2016). Automatically separating picture components from field-captured photos is a provocative and complicated issue for which solutions have only been discovered in recent times (Zhang et al. 2018). A screen behind the leaf before image collection can simplify automatic segmentation (Pethybridge and Nelson 2015; El Jarroudi et al. 2015; Shrivastava et al. 2015), but this increases the time and difficulty of image acquisition. Due to this, most techniques that employ photos acquired in the field need the user to segment the leaves manually (Barbedo 2014, 2016b, 2017).

2.5.4.3 Actual Values

It is not simple to evaluate measurements acquired using the VIS image analysis. Different methods (such as through image analysis technique (El Jarroudi et al. 2015; Peressotti et al. 2011), visual estimation of the specialists, or sometimes some other methodologies (Martin and Rybicki 1998)) can be used to create the "gold standard" reference. Because of subjectivity, even manually depicted picture analysis might hold onto rater error, and in this manner, the systems established rely upon

the references they are entrusted to emulate; they could differ if other "gold standard" references were utilized.

2.5.4.4 System Limitations

Despite the effectiveness of specific cutting-edge methods, such as deep learning, pictures in the visible range occasionally lack the information necessary to discriminate between severity classes. Combining several imaging techniques in these circumstances could be a workable approach (Berdugo et al. 2014), possibly at the expense of increased prices and decreased mobility.

2.5.5 Prospects of Image Analysis for Plant Disease Estimation

Due to the advancement in technology and the more flexibility and capability of software toward the differentiation of diseases, the use and application of image analysis techniques have been continuously increasing in the field of disease assessment. Over the last three decades, the image analysis technique has turned out to be an extensively used disease severity estimation research tool. This technique of disease estimation will also be used in the future as well and provide a rising role in plant disease evaluation due to its potential for trustworthiness and accuracy.

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Remote Sensing: A New Tool for Disease Assessment in Crops

3

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Abstract

The continuous presence of plant pathogens, pests, and diseases affects the agriculture and forestry sector, which demands the need for highly potential and inexpensive methods for diagnosing and monitoring diseases of plants and pests. Based on the huge data obtained from the sensitive analysis and the various systems based on remote sensing, features related to remote sensing are identified, including VIS-NIR, sensitive analysis, characteristics of habitats, thermal and fluorescence parameters, and image- and landscape-based features. However, suitable areas for sensors have been incorporated for the precise and early detection of particular diseases, including field systems, screening for resistance, and assessing and evaluating reactions based on plant defense. Moreover, based on different sensors, remote sensing techniques vary significantly. These techniques assist in the detection and diagnosis of various major diseases. This chapter focuses on RGB camera, hyperspectral imaging, fluorescence spectroscopy, thermography, and multi-temporal-based remote sensing techniques used chiefly on diseases that significantly impact the agriculture economy.

Keywords

Remote sensing · Imaging approaches · Features for plant diseases monitoring · Early disease detection

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3.1 Introduction

There is a need to enhance the yield of agricultural commodities to manage the requirement of the increasing population. It will not be possible to manage these requirements without the application of modern technologies (Mahlein et al. 2012a). Several abiotic and biotic factors reduce the yield of agricultural products such as viruses, fungi, animals, and arthropods. All these may be managed to some extent, but the losses due to abiotic factors may be significantly high. The estimated crop losses due to pathogens (oomycetes, viruses, bacteria, and fungi) are 16% worldwide (Oerke 2020). Monitoring plant pests and diseases at the right time is an important factor in solving these problems. In the last 80 years, the accuracy of visual disease assessment depends upon the conventional disease scales. Identifying the visual symptoms is most important for the accurate diagnosis of diseases. The assessment of the diseases with the emerging technologies gives accurate, reliable, and precise results.

During the last 30 years, the use of digital images and visible light imaging analysis increasing with time. The use of this software has become user-friendly and more sophisticated. For resolving the issues of disease management, remote sensing techniques show significant potential (Mahlein et al. 2018). The data obtained through remote sensing can recognize the crop conditions involving diseases and give information about the most effective strategy for management of diseases (Weiss et al. 2020; Liu et al. 2005). The meaning of the word "Remote" is far away. That's why meaning of the "remote sensing" is the sensing of the objects from a distance. The term "remote sensing" is described by the ASPRS (American Society for Photogrammetry and Remote Sensing) as the technology, and science of gaining precise information about that thing which have physical appearance as well as about the environment, through the process of recording. It is also defined as the science and art of gaining information about a thing without establishing any physical contact. The procedure of remote sensing may be satellite, ground or aerial based (Gogoi et al. 2018). The remote sensing technology has two types.

1. Active (such as radar and LidDAR)
2. Passive (e.g., optical) remote sensing

In active remote sensing, the reflection of the emitted signals from the object is measured on the sensor and the object is irradiated from an artificial source of energy, e.g., radar.

In the passive remote sensing, the natural source applied to irradiate the object, for example, solar radiation, to detect the desired phenomenon.

In the passive remote sensing, there are different imaging cameras and detectors which are used to measure the reflection of the solar radiation that have wavelength of thermal infrared (3–15 μm), NIR (near infrared: 700–1100 nm) VIS (visible spectrum: 400–700 nm), and SWIR (shortwave: 1100–2500 nm), while in active remote sensing, the instruments used are LIDAR and Radar (Fahey et al. 2021).

The passive remote sensing is classified into two categorizes on the basis of using spectral resolution of sensor.

1. Multispectral remote sensing
2. Hyperspectral remote sensing

The use of hyperspectral remote sensing shows good potential in the passive remote sensing because it is non-destructive and non-invasive tool to monitor the abiotic and biotic plant stresses (Jones and Vaughan 2010). Hyperspectral imaging techniques offer various options to observe the disease at the initial stage, through the provision of early indicators in the form of minute variations in spectrum reflectance as a result of reflection or absorption. The small differences in canopies, individual plant or soil, can be more easily seen in hyperspectral images since they can provide comprehensive spectral profiles with hundreds of spectral bands. Therefore, hyperspectral images are used to resolve the large problems for timely and accurate determination of the physiological conditions of the crop. As a result of early detection, the outbreak and spread of the pests may be avoid on the important crops and it also reduce the consumption of pesticides which is harmful for the humans and their environment (Lucieer et al. 2014; Gonzalez-Dugo et al. 2015).

3.2 History

The development of the camera over than 150 years ago led to the establishment of advanced remote sensing technologies. The practice and concept of looking down at the Earth's surface first developed when photographs from cameras attached to balloons were taken for the purpose of topographic mapping in the 1840s. Remote sensing (by application of visible spectrum) is started in 1909 in the form of aerial photography. In 1931, color infrared photography was started, and then it is widely used in forestry and agriculture. In 1950, Ms. Evelyn Pruitt first used the term "Remote Sensing" in the United States. Satellite remote sensing originally started as a dual method to photographing surfaces utilizing different sensors from spacecraft in the early stages of the space age (both American and Russian projects). In 4th October,1957, Soviet was the first who launched man-made satellite named as "Sputnik 1" (Choudhary et al. 2022). In 1956, Colwell used the remote sensing technique to monitor the stem rust of wheat.

3.3 Remote Sensing Techniques on the Basis of Different Sensors

3.3.1 Imaging Approaches

3.3.1.1 RGB-Imaging

To assess the plant health, digital photography is an important technique in plant pathology. Red, green and blue images are called as RGB digital images in short form. The digital cameras are the simple source of red, green and blue (RGB) digital

images for quantification, detection, and identification of diseases and they are easy to operate. The technical factors of these simple digital cameras such as spatial resolution, digital and optical focus, or light sensitivity are increasing with the passage of time. In these days, nearly everybody carries a smartphone or tablet computer, together with a contemporary, powerful digital camera sensor, whether they are phytopathologist or a farmer. Alternative tools for analyzing digital photographs of various plant parts, from inflorescences to roots, include scanners or video cameras. Throughout the growth season, RGB sensors are utilized to monitor plants at every resolution scale.

The biotic stress of the plants have been detected by the using of RGB images with green, blue and red channels (Bock et al. 2010), including the data about the colors in LAB (A and B used for the adversary magnitudes that depend on the non-linear coordinates and L stands for lightness), RGB, HSV (stands for hue saturation value) color, YCBR (Y is the component of luma) and CR and CB are red and blue difference parts of chroma, respectively, and their special arrangement gives the information about the diseases of plants (Bock et al. 2010). Furthermore, variables for the detection and identification of plant disease signs include gray levels, colors, texture, connectivity, form factors, and dispersion (Neumann et al. 2014; Camargo and Smith 2009).

Many research teams have utilized machine learning and pattern recognition techniques to detect and diagnose diseases of plants by using RGB photos (Camargo and Smith 2009; Neumann et al. 2014). Additionally, the classification accuracy is improved by carefully choosing essential characteristics from the RGB pictures (Behmann et al. 2014). In the assessment of plant diseases, the analysis of digital images is important technology. There are different software packages such as "Leaf Doctor," custom-made modules, ASSESS 2.0 and Scion image software are available (Pethybridge and Nelson 2015; Bock et al. 2010; Wijekoon et al. 2008; Tucker and Chakraborty 1997). In ASSESS 2.0, the histograms used as the foundation for the following thresholding are used to assess the color information of the photos. The user may modify the parameters for healthy and unhealthy regions through a well-organized graphical interface. Additionally, once the background has been removed from the item of interest, disease severity can be determined as a percentage or as infected pixels. The measurement of disease severity on single leaves and precisely ordered photos is particularly practical with ASSESS 2.0. The picture acquisition stage needs special consideration. Illumination, uniform focus, and sharpness are important for reliable and for getting accurate results through the analysis of images. Under the natural status, the quality of the image is dependent on the distance between the sensor and the object, pixel size, and the leaf orientation or image angle. Poor picture quality and heterogenic situations frequently lead to difficulties in recognition and low degree of accuracy.

3.3.1.2 Hyperspectral and Multispectral Reflectance Sensors

The classification of the sensors of spectral reflectance is based on the resolution of spectral (such as width and number of the bands which are measured), type of detector (such as non-imaging and imaging sensor), and their spatial scale. The

earliest spectrum sensors developed were multispectral ones. Typically, these sensors evaluate an object's spectral information across a number of somewhat broad wavebands. For instance, data from multispectral imaging cameras may be provided in the G, B, and R wavebands as well as in an extra near infrared band. The development of current sensors in hyperspectral imaging tools with a spectral range between 350 and 2500 nm and a potential of the spectral resolution below 1 nm enhanced the complexity of the measured data (Steiner et al. 2008). Sensors of hyperspectral imaging tools give both spatial and spectral information for the observed things, in contrast to non-imaging, which average the spectral information across a specific region.

Large matrices containing spatial *y*- and *x*-axes and spectral data as reflectance intensity for each waveband in the 3d space, *z*, can be used to show hyperspectral data. The distance between the sensor and object has a significant impact on the spatial resolution. That's why, spaceborne or airborne, far range system contains low level of spatial resolution as compared to the microscopic or near range systems. The spatial resolution has significant role in the detection of plant–pathogen interaction and in the identification of plant pathogens (West et al. 2003; Mahlein et al. 2012b). Field patches that are affected by the soilborne pathogens can precisely detected by the airborne sensors (Hillnhütter et al. 2011) or at the later stage of the disease (Mahlein et al. 2012a; Mewes et al. 2011) or the identification of single symptoms or infected plants and leaves, sensors with a spatial resolution of around 1 m are scarcely acceptable; proximal sensor system is preferred in these situations (West et al. 2003; Oerke et al. 2014). Despite numerous studies, the use of innovative hyperspectral imaging technology to plant pathology and the evaluation of disease severity is still in the early stages of development (Bock et al. 2010). The optical properties of the leaves are described by the absorbed light by the chemicals of leaves (sugars, water, amino acids, and lignin) reflection of light through leaves surface and from their internal structures and transmission of light through a leaf.

Therefore, reflection of light from the plant surface is a complex procedure that depends upon the many biochemical and biophysical interactions. The SWIR (1100–2500 nm) depends upon the composition of water and leaf chemicals, VIS (visible range 400–700) is influenced by the pigment content of the leaf, and NIR (near infrared reflectance 700–1100 nm) mainly influenced by the leaf structure, absorption by the water of leaf and internal scattering process of the leaf (Jacquemoud and Ustin 2001; Carter and Knapp 2001). When a highly specific plant disease or plant pathogen causes changes in reflectance, such as the sequence of necrotic and chlorotic tissue or the presence of typical fungal structures like powdery mildew conidia and hyphae or uredospores of rust, those changes can be described by difficulties in the structure of leaf and chemical composition of the tissues.

During the infection, the obligate fungus parasites such as rusts and powdery mildews have comparatively low effect on the chlorophyll composition and tissue structure as compared to the other pathogens such as, pathogens that are cause of leaf spot, mainly cause the degradation of tissues due to the production of pathogen-related specific enzymes or toxins and their results is in the form of necrotic lesions.

In opponent, rusts and powdery mildews fungi form their structures on the leaf that can induce the optical characteristics of plant–pathogen contact (Ul Haq and Ijaz 2020a, b). By using semi-thin parts of diseased leaf portion and raster electron microscopy, complex and distinctive relationships for sugar beet leaf diseases are exemplarily observed. The highly distinctive and unique pattern of the disease enables the recognition of the disease based on the spectral characteristics of the plant. It is described that the variation of foliar pathogens depends on the reflectance from the leaf in the case of sugar beet (Mahlein et al. 2010, 2013). Based on these studies, Rumpf et al. (2010) were capable to identify sugar beets that were infected with rust, powdery mildew, and Cercospora leaf spot before any typical symptoms appeared. Non-invasive spectral data have been beneficial for monitoring *Fusarium graminearum* in wheat and other plant–pathogen systems (Bauriegel et al. 2011), *Phytophthora infestans* of tomato (Wang et al. 2008), or *Venturia inaequalis* in the apple (Delalieux et al. 2007). It was also shown that hyperspectral imaging technique to be helpful in the assessment of pathogens that producing toxins in the maize crop (Del Fiore et al. 2010).

Additionally, in-field spectral imaging was employed by Bravo et al. (2003) for the early identification of wheat with yellow rust infection, Hillnhütter et al. (2011) effectively differentiated between soilborne diseases by examining the symptoms brought on by the soilborne fungus (*Rhizoctonia solani*) and nematode *Heterodera schachtii* in fields of sugar beet. Apan et al. (2004) used EO-1 Hyperion hyperspectral imaging technique to identify orange rust on sugarcane. Later, Huang et al. (2007) used aerial hyperspectral imaging and ground-based spectral measurements to identify yellow rust in wheat crop. Hyperspectral imaging technique is also widely applied for the observation of fruit quality and health along with the identification of plant pathogens during the vegetative period of the crop. By using hyperspectral imaging tool, defects on the surface of apple (Mehl et al. 2004), rot disease of strawberries (ElMasry et al. 2007), and canker lesions on the citrus fruit can be identified (Qin et al. 2009). These methods are useful for avoiding storage infections of crops and screening fruits.

3.3.1.3 Thermal Sensors

IRT stands for infrared thermography which measures temperature of plants and has relationships with crop microclimate, variations in transpiration rate caused by early plant–pathogen infections, and plant water status. Infrared and thermographic cameras are able to detect infrared radiation that is emitted in the thermal infrared range between 8 and12 mm, which is represented in fake color photographs with each image pixel including the value of temperature of the observed thing. Infrared thermography may be used at the different spatial scales and temporal from small scale to airborne applications. However, it is mostly depending on the environmental conditions such as sunlight, wind speed, rainfall, or ambient temperature. The transpiration of the plant shows close relationship with the leaf temperature, which is affected in different ways by the variety of pathogens. While many other foliar pathogens such as rusts or leaf spots bring well defined and local changes, damaged

by the plant root pathogens such as *Pythium* spp. or *Rhizoctonia solani*, or systemic infection that mostly induce water flow in all parts of plants and transpiration rate.

It is reported that the temperature changes in plants are due to the infection caused by the pathogens or due to the defense mechanism of the plant. By using IRT technique, successfully monitored the downy mildew of cucumber (*Pseudoperonospora cubensis*) or apple scab (*Venturia inaequalis*). The variability between and among leaves can be used for efficient IRT image analysis. A major predictor of the emergence of plant disease is the mean temperature differential within individual leaves, crops, and plants.

3.3.1.4 Fluorescence Imaging

Different parameters of chlorophyll fluorescence have been used in the determination of plant photosynthetic activity. Chlorophyll fluorescence imaging devices are frequently active sensors that measure photosynthetic electron transport using laser light or LED source. Fluorescence imaging is used to evaluate variation in plant photosynthetic system due to the abiotic and biotic stresses on the leaf surface. Combining the image analysis and fluorescence imaging techniques to be useful in the quantification and determination of fungal infection. In order to assess plant disease at the field or canopy level, research has focused on collecting fluorescence characteristics from sun-induced reflection in the field (Mahlein 2016).

3.3.2 Non-imaging

3.3.2.1 Vis-NIR Spectroscopy

Visual infrared-near infrared spectroscopy (VIS-NIR) is a rapid and non-destructive procedure which provides prediction of biological and chemical composition of the system. The Vis technique of the spectroscopy can examine the pigment and color analysis, while NIR is used to measure the quantity of macro components, mostly water. The visible region that ranges between 400 and 700 nm can provide data on the basis of spectral features of pigments and it is also used during the photosynthesis process of the plants. In these pigments, anthocyanin, carotenes, and anthocyanin are involved that induce the color appearance and can show disease in trees and plants. Each pigment has specificity in spectrum absorption such as the green region absorbs 530–550 nm wavelength light which is due to the presence of anthocyanin, and due to the presence of carotenes, range of the light wavelength is 420–503 (Zahir et al. 2022).

3.3.2.2 Fluorescence Spectroscopy

Fluorescence spectroscopy is a procedure which is applied to determine the fluorescence of the substance after reflection along a beam which is usually ultraviolet and having wavelength 10–400 nm. The fluorescent material has unique color that only visible when comes in the front of UV light since the absorbed light is undetectable to the human eye while the released light is in the detectable range. The mechanism of this procedure is involved using a light beam that induce the electrons in

molecules of a substance and as a result light is emitted. FS has wide range of applications. FS is used to monitor the stress levels and physiological states of the plants. Generally, the use of FS tool in plant study, especially in nutrient deficiencies and plant diseases has gain more attention. The FS procedure applied as a tool determines and senses the pathogens of plant at their initial stage. For instance, FS has been used to determine the plant–pathogen interaction in leaf rust and powdery mildew of wheat, spring barley, to identify the cucumber disease, and to quantify and identify the infection of banana due to the *Fusarium oxysporum* f. sp. *cubense*.

There are several devices of FS that have used to observe and gain information from plant specimens. In these devices, fiber optic fluorescence spectrometer, portable multiparametric sensor, and imaging multispectral fluorescence are involved. However, the most reliable and accurate FS device to detect plant pathogens is the fiber optic fluorescence spectrometer in contrast to other devices. There are four emitted wavelengths used in the FS, that are Green (G), Red (R), Blue (B), and UV and the excited FS is observed in red RF, far red RF, and yellow YF.

3.4 Remote Sensing Features for Plant Diseases and Pest Monitoring

Agriculture and the forest sector are highly threatened by the ongoing progression of plant diseases and pest attacks globally (Oerke 2006; Strange and Scott 2005). Plant protection practices must be guided by knowledge about the location, scope, and severity of disease and pest incidence. Techniques and approaches based on remote sensing (RS) might be a prime addition that has the capability to monitor and diagnose diseases on plants as well as on pests, an extensive scale because standard field searching of pests and diseases on plants is quite effortful, liable not to be impartial, and typically exhibits minimum proficiency (Mahlein 2015). Methods of "radiodiagnosis" of plants that effectively provide contact-free and at a global level constant monitoring and examining of pests and diseases on plants that could be viewed in the remote sensing of pests and diseases on plant diseases.

With regard to the efficient detection or monitoring of pests as well as diseases, numerous systems of RS are accessible that can be applied efficiently to possibly monitor the progression of the disease. These RS systems provide data collection ranging from gamma-ray to microwave radiations, working with both radiations that are either passive or active. Many struggles have been brought to action for various RS systems in detecting the infection symptoms such as blights, pustules, and scabs, structural changes such as landscape structure, canopy structure, and physiological responses such as changes in water content, pigment content, caused by pests and diseases on plants in order to effectively detect and monitor pests and plant diseases (Hahn 2009; Sankaran et al. 2010; Mahlein 2015).

It is essential to recognize useful and distinguishing RS characteristics and features in order to employ RS-based observations in monitoring and examining pests and plant diseases. Until now numerous RS-based features and attributes have been suggested or discovered for identifying plant pests and diseases as well as for

identifying their natural environment. The primary RS-based features involve features based on the landscape as well as image-based features for thermal and fluorescence systems, and VIS-NIR spectral characteristics. Considering that different diseases and pests have different damage processes, a precise and accurate assessment of RS characteristics is typically required to provide efficient monitoring (Mahlein 2015).

3.4.1 VIS-NIR Spectral Features

VIS-NIR spectral features are extensively employed since VIS-NIR sensors are considered widely used systems based on RS for tracking pests and plant diseases. The most basic version of these properties is band reflectance. The majority of researchers have found that several diseases of plants and pests are sensitive to the green, red, and NIR spectral areas. There are several approaches to altering the values of spectrum reflectance, including continuous wavelength transformation, spectral derivatives, and continuous removal transformation.

Spectral derivative transformation is generally executed to apprehend convex features of the spectral measurement of plants which includes red edge position and amplitude.

The continuous removal technique is usually used for the quantitative characterization of both the convex and concave traits of the plant's spectra performed along the spectrum in a precise absorption location, which can be employed efficiently for the diagnosis of many plant diseases mainly wheat powdery mildew and tomato leaf minor (Xu et al. 2007; Zhang et al. 2012).

Continuous wavelet analysis or CWA has been employed for the identification and detection of many pests and diseases on plants (Cheng et al. 2010; Luo et al. 2013). CWA-derived features outperform certain traditional spectral-related features in identifying winter yellow rust of wheat due to their ability to capture some subtle changes in spectral shape (Zhang et al. 2014).

3.4.2 Fluorescence and Thermal Parameters

For the diagnosis and identification of numerous pests and diseases in plants associated with thermal and fluorescence sensors, some variables and parameters have been set to create an efficient linkage between the symptoms of infection and signals based on thermal and fluorescence sensors. For the diagnosis of the presymptomatic state of a pathogen, some researchers have used the ratio by dividing the fluorescence amplitude such as F686/F740 on the basis of continuous fluorescence spectra with the addition of fluorescence peaks (Bürling et al. 2012; Tartachnyk et al. 2006; Kuckenberg et al. 2009). Additionally, a number of fluorescence parameters were used in the detection of pests and plant diseases based on the saturation pulse fluorescence analysis, including the non-photochemical quenching

(NPQ), the highest potential in light-adapted material of PSII photochemistry (Fv'/Fm'), the efficiency of photosystem II (PSII) in terms of quantum yield and the maximal quantum efficiency of primary photochemistry photosystem II (PSII) (Fv/Fm) (Cséfalvay et al. 2009; Scholes and Rolfe 2009; Iqbal et al. 2012). Characteristics which are produced from sensors based on thermal infrared for detecting pests and diseases on plants are reasonably easy to understand and less complex than spectral features and fluorescence features. In essence, the canopy and leaf temperatures have been considered quite significant signals. The differential temperatures between the air and leaves (Tleaf − Tair) in a controlled environment such as a greenhouse were discovered to be useful in identifying presymptomatic *Plasmopara viticola* infection in grapevines (Stoll et al. 2008).

3.4.3 Image-Based and Landscape Features

For monitoring and surveying diseases on plants and pests, imaging observation can be obtained and examined based on distinct systems of RS such as fluorescence, thermal and VIS-NIR systems. Using the features such as texture and landscape, the precise evaluation of the severity and in-depth disease mapping of the area where infection occurs can be done efficiently (Bauriegel et al. 2011). To extract texture features from images, including uniformity, variance, mean intensity, correlation, contrast, modus, product-moment, entropy, inverse difference, and information correlation, the colour cooccurrence method (CCM) is frequently used which are crucial especially at the micro (leaf) level for identifying plant diseases and pest (Donohue et al. 2001; Shearer and Holmes 1990). Yao et al. (2009) suggested the classification of rice blast, rice bacterial leaf blight, rice sheath with 97.2% of precision based on the group of shape and texture traits. Some spatial measurements such as landscape features bring out from the RS images. Moreover, the features based on the texture of image can identify the pattern based on spatial and geographic distribution for pests and plant diseases and provide helpful information in their extensive such as regional and plot monitoring. In addition to the features based on optical, thermal, fluorescence and last but not least image-based offers a different viewpoint for keeping track of pests and plant diseases.

3.4.4 Features Associated with Habitat Characteristics

A few attempts have been done to use data based on RS to define the suitability of the habitat of the pests and the plant pathogens, irrespective of the traits of RS that cannot be indirectly associated to losses brought on by pests and plant diseases. Given that stressed plants are frequently more vulnerable to disease and insect attack, the Tasseled Cap Transformation (TCT) based metrics such as brightness, greenness, and wetness linked with soil moisture and vigor and vitality of plant were found to be an effective substitute for habitat fitness (Coops et al. 2009; Zhang et al. 2013; Wolter et al. 2008). Regardless of the metrics based on TCT, LST, and Vis, are

effectively employed to give substantial and vital information for habitat state description. In the efficient monitoring and observation of mosquitoes larvae, tree woolly adelgid, wheat powdery mildew, and spruce budworm, several VIs such as MSI, WI, PRI, SAVI, MSAVI2, NDWI, and TVI connected with biomass, water contents, and concentrations of pigments are highlighted (Brown et al. 2008). The land surface temperature which is used to monitor the rated intensity of transpiration in plants is easily accessible from products obtained from satellite imagery such as MODIS-LST or it can be recovered from many satellites of thermal bands such as HJ-IRS, Landsat TM, and ASTER. Suitable habitat for the monitoring of disease and pests can be obtained with 82% of verified precision. Assimilation of RS features and parameters of meteorology together constructed the disease forecasting model (Zhang et al. 2013). An accuracy of 69–78% of their results suggested that adding up information related to RS efficiently improves the level of precision for plant disease and pest monitoring.

3.4.5 Sensitivity Analysis for Selection of Features

It is a prerequisite step always that is required to do a sensitivity evaluation to determine the best feature of RS for the monitoring and examination of pests and diseases on plants since they may produce noticeably distinct symptoms and differing environment characteristics. In order to do this, statistical techniques have been used to evaluate feature sensitivity. Analysis of Variance (ANOVA) and independent *t*-tests were widely employed, for instance, to categorize different types of illnesses and pests or to distinguish between different infection levels (Yuan et al. 2014; Zhang et al. 2012). For RS features identification, the Pearson correlation analysis is considered the most favored and highly sensitive technique of analysis (Huang et al. 2012; Zhang et al. 2012, 2014). Yang et al. (2007) found that the sensitivity band for detecting rice leaf folders drastically changed from tillering stage at 757 nm to the heading stage at 445 nm. Furthermore, it is important to keep in mind that sensitivity features may differ across observation scales.

3.5 Relevant Areas for Sensors in Plant Disease Detection

Plant pathologists have access to a wide range of sensor technologies that may offer high and accurate resolution data on agricultural crops and serve as the foundation for the on-time detection and diagnosis of diseases on plants. It is astonishing how far these technologies have come in the last 40–50 years in terms of development and application to agriculture as well as detection and monitoring of plant disease (Brenchley 1964; Nilsson 1995; Jackson and Wallen 1975; West et al. 2003; Seelan et al. 2003). Latest and customized techniques for solving plant and agricultural science-related problems have been created as a result of developments in plant phenotypes and agriculture (Cobb et al. 2013; Fiorani et al. 2012). The most effective sensors are now being employed for non-invasive field assessments of

crop nutrient status. In agriculture, practical base future applications will benefit from the development of new, low-cost sensor technologies that perform satisfactorily and are now on the market (Grieve et al. 2015). There are equipment and technical options for phenotyping in the field, greenhouse, and other environments. However, they cannot be used widely because these are highly customized and specialized prototypes.

3.5.1 Field Systems

The imaging platform developed by Polder et al. (2014) for the diagnosis and detection of Tulip breaking virus (TBV) which usually infects bulbs of tulip flowers, or the monitoring and detection of the wheat yellow rust caused by *Puccinia striiformis* hyperspectral imaging platform prototype developed byBravo et al. (2003) are two examples of cutting-edge systems with potential field applications. A robot with multispectral sensors and pipelines for mechanized vision online evaluation was created by Polder et al. (2014). There were not many technical specialists available to rate tulip bulbs, which motivated this study. This technique was able to be optimized and adjusted to reach a degree of precision comparable to that attained by seasoned rating experts. Under light ambient conditions, the yellow rust disease of wheat can be able to detect and categorize in fields successfully with a rate of 96% using the hyperspectral imaging "buggy" of Bravo et al. (2003). Their findings are highly promising for the establishment of optical sensor systems that are both affordable and accurate for the early plant disease detection in various crops.

3.5.2 Resistance Screening

Different technological techniques have been created for plant phenotyping. The process of development began with controlled investigation and examination of individual plants (Chaerle et al. 2007; Jansen et al. 2009). Improved field systems have lately quite durable, allowing for a comprehensive evaluation of plant execution and performance across many plots or throughout the whole canopy of plants (Walter et al. 2015). It has been demonstrated that optical sensors can assess the degree of sensitivity and/or resistance of various genotypes and variants to a particular disease. By using multispectral and fluorescence imaging, Chaerle et al. (2007) contributed by distinguishing lines of sugar beet with varying degrees of sensitivity to *C. beticola*. In order to assess the symptoms on *Phaseolus vulgaris* by *Xanthomonas fuscans* subsp. *fuscans*, depends on the chlorophyll fluorescence metrics *Fv*/*Fm* on picture pixels Rousseau et al. (2013). Additionally, more contemporary innovations were produced, such as the HyperART system for concurrently monitoring and observing leaf transmission as well as leaf reflectance (Bergstrasser et al. 2015). In a preliminary application study to evaluate different sugar beet resistance/susceptibility levels to *C. beticola*, this sensor device showed potential. It has been demonstrated that extra data from the measurements during transmission

boosts the sensitivity of detection; nevertheless, the measurement methodology and procedure are fairly complicated and still call for a significant amount of human work.

3.5.3 Assessment of Plant Defense Reactions

The majority of research has been published on the subject of detection and diagnosis of plant disease systems which are dependent on symptoms, biochemical, and the changes that occur physiologically prior to symptoms. In the screening of resistance, pathologists are interested in minute defensive responses that are essential for plants' capacity to pathogen invasion or restrain pathogen growth. Robinson (1969) defined resistance in the host as a plant genotype's capacity to impede a pathogen's growth and/or development. Long recognized as two broad forms of resistance, qualitative resistance is controlled by a single gene (complete or vertical resistance), and qualitative resistance is controlled by numerous genes with partial effects (incomplete or horizontal resistance). A significant range of defensive mechanisms are activated by plants in response to pathogen infection (Glazebrook 2005). These strategies include the synthesis of proteins and antimicrobial metabolites, the formation of callose and lignin to physically strengthen cell walls, and the stimulation of hypersensitive responses. Following the initial encounter with a pathogen, these changes take place at the level of tissue and cell and are necessary for eventually interactions either compatible or incompatible or, when examining the plant edges, for sensitivity or tolerance of a genotype or any disease. It is necessary to build particular sensors with a high and efficient spatial resolution in order to evaluate these extremely early and marginal changes. By integrating highly throughput DNA cloning and transformation techniques based on single cells adjusted with phenotyping and automated microscopy, Douchkov et al. (2013) developed a planned platform known as the "microphenomic." On several genotypes of barley, they were able to evaluate the penetration efficiency of fungus *Blumeria graminis* f. sp. *hordei*.

It is notable that in this instance, histological analysis of leaf portions that had previously been detached and decolored was employed. From digital pictures obtained from microscopical RGB, sites of fungal penetration and as feeding organs powdery mildew may be evaluated automatically and tallied. This extremely intrusive method only allows for the examination of a plant pathogen's interactions with its host plant at a particular time period. Generally, this enables distinguishing various types of resistance such as complete, incomplete, and partial resistance, whereas some portions impede a pathogen's penetration initially, others cause a hypersensitive reaction to reduce supply of nutrients to make the pathogen starved. Moreover, spore forming capability of pathogen is also restricted in some situation (Glazebrook 2005). Kuska et al. (2015) have created a technique for hyperspectral microscopic analysis. With this data-driven phenotyping technique and sensor-based system, evaluation of over time interactions between host and pathogen and the

differentiation of barley genotypes against powdery mildew in terms of varying susceptibilities were made possible on a small-scale basis.

3.6 Use of Different Remote Sensing Methods for Different Diseases

Based on various sensors, for monitoring and detecting pests and diseases on plants, generally remote sensor techniques involve two groups which are discussed as follows:

- **Imaging Approaches**
  - RGB camera
  - Imaging based on multispectral spectrum
  - Imaging based on hyperspectral spectrum
  - Thermal base imaging
  - Fluorescence-based imaging
- **Non-Imaging Approaches**
  - Spectroscopy of VIS and IR
  - Spectroscopy technique based on fluorescence

3.6.1 RGB Camera

3.6.1.1 Use of Airborne Remote Sensing for Plant Disease Detection

3.6.1.1.1 ADAR System for the Detection and Diagnosis of Rice Sheath Blight Disease

Four aerial-based remote sensing photos were collected with the help of the ADAR (Airborne Data Acquisition and Registration) System 5500, Qin and Zhang (2005) in central Arkansas, USA identified the rice sheath blight disease. The pictures were divided into four bands: near infrared was included in band 4 with 780–1000 nm wavelength, red was in band 3 with 610–680 nm wavelength, green was included in band 2 with 530–600 nm wavelength, and blue was in band 1 with a wavelength of 450–540 nm. They compared the photos with the field disease index using three different techniques: the Direct Band Digital Number (DN) value, Standard Difference Indices (SDI), and Ratio Indices (RI). The findings suggested that RI and SDI may be used to remotely identify the pathogen that causes rice sheath blight (Qin and Zhang 2005).

3.6.2 Hyperspectral Imaging

3.6.2.1 Early Detection of Wheat Yellow Rust Disease Caused by *Puccinia striiformis* by Using Hyperspectral Imaging

Hyperspectral base imaging in agriculture has increased significantly in recent years. The amount and information quality become increased by this technology's usage of narrowband sensors. The spatial *Z*-axis, a spectral *X*-axis, and a spatial *Y*-axis are

axes on which whole data is based. The complete information of wavelength is contained in each spatially situated pixel of a picture. For the purpose of early identification of the winter yellow rust disease (*Puccinia striiformis*) in wheat, Bravo et al. (2003) employed VNIR hyperspectral imaging. The full spectral range from 463 to 895 nm was covered by 19 wavebands that were each 23 nm or 30 pixels wide. They noticed that due to the disintegration of leaf structure internally, the symptomatic plants had increased reflectance in the visible spectrum (VIS) due to decreased activity of chlorophyll and in the near infrared (NIR) spectrum higher absorption was observed (Bravo et al. 2003).

3.6.3 Thermography for Plant Disease Detection

3.6.3.1 Thermographic Assessment of Apple Scab Disease

Oerke et al. (2011) injected 6×10^5 conidia/mL of the HS1 isolate of *V. inaequalis* onto apple leaves and monitored the results daily. They captured thermograms using a spectral sensitivity ranging from 8 to 12 m, Varioscan 3201 ST camera and by using IRBIS plus software pictures were then analyzed (Vers. 2.2, InfraTec.). Prior to the development of obvious scab signs, the researchers noticed that the leaves injected with *V. inaequalis* conidia had shown the appearance of concentric spots of very low leaf temperature (6 dpi). When the typical scab symptoms appeared at 8 dpi, the affected leaf area and difference of temperature from non-diseased areas increased, but the temperatures of apple leaves that were not infected varied little over space (Oerke et al. 2011).

3.6.4 Fluorescence Spectroscopy for Plant Disease Detection

3.6.4.1 Early Detection of the Hypersensitive Reaction to Tobacco Mosaic Virus Using Multicolor Fluorescence Imaging

In the "Samsun NN" and "Sumsun nn," the resistant and susceptible cultivars of tobacco plant, respectively, Chaerle et al. (2007) inoculated tobacco mosaic virus to observe the HR response and subsequent death of plant cells aggravated by TMV inoculation. A "Luminescence spectrometer LS52" equipped with a "Xenon flash lamp (FX-4400)" was used to excite the fluorescence. After 88 h of inoculation with TMV observation was started. Above 400 nm of wavelength, ultraviolet light as compared to the control induced blue fluorescence which considerably enhanced during the hypersensitivity response to tobacco mosaic virus infection. Towards the 550 nm range, the difference in fluorescence steadily diminished. The susceptible tobacco cultivar "Samsun nn" was not changed substantially in fluorescence emission by TMV infection (Chaerle et al. 2007).

3.6.5 Multi-temporal Remote Sensing for Plant Disease Detection

3.6.5.1 Use of Multispectral Remote Sensing for Multi-temporal Wheat Disease Detection

For the identification and detection of wheat leaf rust and powdery mildew, QuickBird satellite multispectral multi-temporal images with high resolution were used which are evaluated by Franke and Menz (2007) in Bonn, Germany. Mixture tuned matched filtering (MTMF) and NDVI techniques were used to classify the data. The initial scene's classification accuracy was 56.8%, but the subsequent scenes attained much better accuracies of 65.9% and 88.6%, respectively, as compared to the initial scene of classification accuracy (Franke and Menz 2007).

3.7 Conclusion

For disease and pest management, large-scale growing of agricultural crop is the need of the hour for early disease diagnosis. In order to monitor and predict epidemics, remote sensing offers precise, non-invasive, accurate, quick, and reliable estimates of diseases. When used to identify illnesses in green plants, data obtained by hyperspectral remote sensing is collected from low altitudes and often has a great spectral and spatial resolution. Regional agricultural disease mapping has proved to be quite efficient for multi-temporal data based on remote sensing. The approach of categorization based on spectra can be used to identify agricultural diseases. When comparing the NDVI spectral profiles of healthy and diseased crops, a significant difference could be seen that indicated situation of crops under stress. To dramatically spatialize diagnostic results, remote sensing technology will be very beneficial. This will increase agricultural sustainability and safety by reducing the costly usage of pesticides for crop improvement and protection. A multidisciplinary approach involving engineering, plant pathology, and informatics is necessary to fully use the promise of these highly advanced, new technologies. The adoption and complete awareness of this approach will be enhanced by a solid decision support system via cross-disciplinary collaboration.

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Image Analysis and Processing Approach: An Automated Plant Disease Recognition Technology

4

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Abstract

Plants disease development mainly depends on important factors like the plant, pathogen, and suitable environment. The presence of inoculum in the vicinity of the plant is very important. And inoculum comes in contact with the plant through water, soil, insects, and air. After physical contact with the pathogen, the plant responds to the pathogenic presence in multiple ways. Evaluation of losses due to pathogen attacks is always important, and assessment is made for mitigation purposes. Conventionally diseases are assessed through a predetermined rating scale. The rating scales are devised individually for each case depending on the nature of the pathogen and organ or tissue under attack. Some abiotic stresses also cause disease-like symptoms which could be differentiated by an expert only. It is important to know about the concept of disease, disease development, and plant resistance responses before image-based disease assessment.

Keywords

Disease image analysis · Symptom analysis · Imaging · Machine learning · Image processing

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4.1 Basics of Plant Disease Development

Animals have a highly advanced blood vascular system that plays many functions in their life, including defense against diseases; moreover, the animals can escape the pathogen populated sites by physical movement. Plants, contrary to animals, are sessile and are exposed to bacteria, fungi, viruses, nematodes, etc., which may or may not cause disease in plants. There is a continuous battle between the plant host and the concerned pathogen in their periphery. The pathogen's ability to cause disease is not so simple; they have to supersede many barriers set by nature in plants (Ahuja et al. 2012). Depending on their dispersion method, the pathogens enter the plants through roots or aerial parts. Their entry is subjected to the natural wounds and/or their ability to overcome the physical barriers through enzymatic digestion. To achieve such aggression, they need cell wall degrading enzymes to interfere with the normal functions of the plants.

Since thousands of microbes surround plants, many of which are pathogenic to the plant or other plant species. Some microbial pathogens are highly specific and cause infection to specific plant types are called narrow host range pathogens, while other pathogens that can cause infection on a reasonable number of plant species are called broad range pathogens (Ashutosh Rai 2014; Ul Haq and Ijaz 2020). But plants are immune to the majority of pathogens.

Similarly, the plants having pathogen-specific resistance is called host resistance, while resistance of plants to a broad range of pathogens is called non-host resistance. The critical step is the compatibility or recognition of the pathogen both for host and non-host plants. An incompatible plant–pathogen interaction between a pathogen and non-host plant involves the induction of several responses like signaling compounds, cell death of the infected region, and appearance of an increase in the level of reactive oxygen species to hypersensitive response, which is an important type of plant defense (Zheng et al. 2012). Non-host resistance is either indicated by no visual symptom or necrosis of the localized region. From this discussion, it is clear that non-host resistance results in no visible symptom or strong delayed response like necrosis of the limited localized infected part. These two possibilities are dependent on the species of pathogen and non-host plant. A pathogen not showing symptoms on one non-host plant can cause localized necrosis on the other plant species.

Plants and animals possess an innate immune system against pathogens, while vertebrate animals have an adaptive immune system that produces specific antibodies against specific antigens released by the microbial pathogens. The plant's innate immune system consists of constitutive and inducible defense mechanisms. The constitutive resistance mechanisms include physical barriers formed by the plant cell wall and preformed low molecular compounds, peptides, and proteins. The inducible defense system against microorganisms relies on detecting unique patterns known as Microorganism/Pathogen Associated Molecular Patterns (MAMPs/PAMPs) specific to these organisms. When microorganism interacts with plants, these MAMPs such as peptidoglycans, lipopolysaccharides, and flagellin are sensed

by pattern-recognition receptors (PRRs) of the plant to instigate the induced resistance response (Zhang et al. 2013).

These PRRs have a similar leucine-rich repeat (LRR) as animal PRRs. In Arabidopsis, the transmembrane flagellin receptor FLAGELLIN-SENSITIVE 2 (FLS2) is one example. It is located in the plasma membrane and recognizes the bacterial flagellin epitope, which is a bacterial MAMP. The interaction of bacterial MAMPs and PRRs from plants results in different plant responses, such as the production of phytoalexins, PR proteins, and antimicrobial peptides (AMPs), and involves the signaling compound i.e., salicylic acid (Newman et al. 2013). AMPs are widespread in plants and animals, and several groups of these peptides have been described from plants, such as thionins, plant defensins, lipid transfer proteins, snakins, and hevein-like peptides. These plant AMPs are basic and contain several cysteine residues forming disulfide bridges.

The immunity developed under the involvement of these receptors in plants is called pattern-triggered immunity (PTI). Microorganisms try to overcome PTI by delivering effector molecules into the plant cell. One of the best-understood examples of bacterial effectors is AvrPto produced by *Pseudomonas syringae* pv. *tomato*. These effector molecules suppress the plant's pathogen-triggered immunity (Altenbach and Robatzek 2007). However, in response to these effector molecules, plants have evolved resistance (R) proteins, mainly intracellular immune receptors. These molecules can recognize pathogen effector molecules, either directly or indirectly, and the result of this resistance response is called effector trigger immunity (ETI). The important outcome of ETI is the hypersensitive response, a form of programmed cell death (Article et al. 2014). During the hypersensitive response, the infected cells start to die, and this is an effective resistance mechanism against biotrophic pathogens which rely on living plant cells to obtain their nutrients.

Plants communicate the onset of disease to alert the other healthy parts of the plant. These uninfected parts become more resistant to infection by different pathogens. This resistance is called systemic acquired resistance (SAR). The induction of SAR is followed by effector-triggered immunity, and this immunity is effective against a broad range of pathogens (Durrant and Dong 2004).

4.2 Structural Insight Is Important

The cuticle is the outermost physical structure made up of the epidermal cells of plant organs in the phyllosphere. The cuticle is coated with wax, or the latter is intermingled in minute amount as a surface layer or an irregular manner. This is a natural barrier against biotic and abiotic stresses. In the case of biotic stresses, it deprives the pathogens, especially fungal spores, of the availability of moisture due to the presence of wax which lacks affinity for water. In the case of bacteria, it reduces their adhesion and multiplication probability by providing an escape from pathogenicity due to hydrophobicity. The uppermost cuticle having wax is called cuticle proper. Cuticle proper lies exactly on the newly formed cuticle layer adjacent

to the cell wall (Pogorelko et al. 2013). The thickness of the cuticle varies among plant species depending upon the organ containing it. The cuticle is composed of cutin polymer.

Biosynthesis of the cuticle from the epidermal cell is complex; more than one gene is involved in this process, and these genes are reported in more than one plant species which shows their conserved nature, while most of the work is reported in the model plant *Arabidopsis thaliana*. Chemically, the cutin is an ester of 16–18 carbon-containing fatty acids and glycerols. Esterases divide the cutin polymer into comparatively smaller monomers. Wax is chemically ester of alcohol and much longer fatty acids (containing up to 40 Carbons) or the various derivatives of the same. Epicuticular wax gives the surface insulation while the intracuticular supports the structure from inside.

4.3 Pathogen Assessment at the Surface

In the case of fungal spores containing an average or basal level of cutinase enzyme, upon contact with plant surface, disintegrate a very small proportion of surface cuticle into monomers. The presence of these monomers in this interaction further activates mass production of the enzyme mentioned above inside the pathogen cells, linked to emergent transport or secretion to the interaction point for the further decay of the cuticle. Thus induction of this high level of cutinase is mediated through plant monomers. These monomers further activate the adhesion of spores and develop a specialized infection structure, "appressorium," as in the case of rice blast fungus *Magnaporthe grisea*. As already mentioned, wax is the critical component in the formation of cuticle proper, and the cuticle adjacent to the cell wall also plays a role in the induction of the fungal pathogenicity process. The wax on mango fruit and leaves is used to induce the germination and development of infection structure in *Colletotrichum gloeosporioides* fungus. Wax is isolated by chloroform as the solvent for this chemical; the in vitro application of wheat leaf wax enhances the formation of infection structure in the wheat rust fungus *Puccinia graminis* f.sp. *tritici*.

4.4 Explanation of Plant Defense

The monomers of plant cuticle as the degradation product of the pathogen's digesting enzymes are perceived as primary signals for the host to prepare for necessary defense. Simply these monomers act as primary elicitors for the plant. But the effective concentration of these monomers is still debatable how it can be fixed that a particular type and concentration is considered minimal for the activation of plant defense. Additionally, their presence at the interaction site is a well-established fact for such plant reactions. External application of cutin monomers present in the hydrolysates of apple or tomato on Cucumber used to induce the production of reactive oxygen species in the host as a result of induction. Similarly,

Rhizoctonia solani infects bean leaves, the spores in water used to cause disease while the same spore suspension aided with cutinase derived from *Venturia inaequalis* resulted in an apparent reduction in symptoms of disease in a comparative study.

The cell wall is the outermost structure of the cell. It can be divided into three important parts. The middle lamella is present as the adjacent structure of the neighboring cells. The other two structures are the primary and secondary walls. The primary walls lie outside of the secondary wall. The secondary wall is made of lignin and is present mainly in xylem cells, thus preventing the loss of water during transportation. In the absence of a secondary wall, the primary walls remain in contact with the cell membrane, which is present as the living structure of the cell surrounding the cytosol and organelles. Both of these walls vary in composition and thickness. The ingredients from which a cell is formed are mainly cellulose, hemicellulose, lignin, glycoprotein, proteins, enzymes, and inorganic ions in minor amount. The ingredients are synthesized inside the cell and or in the endomembrane system of the cell as insoluble particles and assembled to form this rigid structure. The involvement of various proteins is reported, as more than 2000 genes directly or indirectly control the synthesis and activity of plant cell wall. Most of the plant cell machinery is involved in synthesizing and transporting the ingredients in forming the cell wall.

4.5 Resistant Plant and Disease Severity Concept

The cell provides a barrier during the attack of pathogens. This barrier is not entirely successful, yet it plays a primary role in the defense machinery. The phytopathogens must interact with the cell wall, depending upon the weapons of attack they have acquired during the long evolutionary race with the plant. Almost all necrotrophic fungi and bacteria cause soft rots, and some biotrophic fungi or bacteria primarily use the cell wall digesting enzymes in their interaction with the plant. Pathogens do not equally target all the components comparatively; pectin and xylan, which are primary constituents of the cell wall in cereals and among dicots, and are digested mainly by these enzymes as compared to cellulose which is packed as strong stuff of microfibrils. So these degrading enzymes, pectinases and xylanases, are important virulence factors for necrotrophic pathogens and help them in disease incidence.

To counterattack, the plant produces different inhibitor proteins. In the growing and metabolically active regions, the cell wall stiffness is not an easy target for the pathogen to attack. In the case of soil-borne fungi, it can colonize at the apical region, but the real invasion is at the elongation zone, the point having little weakened cell wall due to little metabolic move. This shows how the cell wall is important as a defense barrier. The fungi and oomycetes establish a special feeding structure called haustoria when they are in contact with the host cell surface. Bacterial pathogens lack such structures, but in the case of gram-negative pathogenic species, they have a specialized secretion system made of proteins to deliver effector proteins into the plant cell through pili. Papillae are also deposited at the

site of infection and act as a physical barrier in penetrating pathogenic structures and products inside the cytosol. These papillae are active sites for the presence of secondary metabolites, which have antimicrobial properties. These are induced structures and appear at the time of infection. In the resistant plant, they evolved quickly as compared to susceptible plant types. In this way, cell wall halt such invasions at an early stage with a low cost of the cell energy compared to cell death which is also a sort of plant defense.

Additionally, the thickness of the cell wall of epidermal cells, which are exposed to pathogens earlier than others, is more significant in comparison. Such cells provide resistance to plants against fungal and bacterial pathogens aimed at direct interference. Maceration of cell wall through enzymatic activity helps the necrotrophic fungi and bacteria to overcome such a stiff structure and successful invasion with varying degrees of disease intensity.

4.6 Defense Induction in the Plant

The cytoplasm is the dense fluid that gives the platform for all activities of biomolecules for the existence and survival of the cell. But in some cases, the cytoplasm acts as a resistant structure by surrounding the hyphae of growing fungi or some granular particles that hinder the further advancement of the fungal structures by breaking them. In some cases, the plant cell nucleus divides, while in the situation of more vulnerable fungi, the cytoplasmic resistance response of the plant is overcome easily by the invading fungi. Enlargement of the nucleus and cytoplasm is also considered as the resistance response of the cytoplasm against pathogens with thick cytosol.

4.7 Callose Production and Image-Based Quantification

The nature of the cell wall as a physical barrier is already briefly discussed. The cell wall also possesses some inducible chemicals and structures which provide a great deal of primary defense against various biotrophic and hemibiotrophic pathogens. These structures include the appositions of the cell wall known as papillae. Necrotrophic pathogens secrete different hydrolytic enzymes, which macerate the effect of these defense responses. These structures are more effective against bacterial and fungal pathogens. Fungal pathogens face hurdles due to the induction of these assemblies during the formation of their feeding structures.

Additionally, these structures are equipped with various chemicals like callose, phenolics, lignins, etc., enhancing the plant cell's overall resistance against microbial invasions. Rapid response in the form of these structures equipped with biomolecules presents the plant's resistance against a particular pathogen. Some of the reports on the callose are β-(1,3)-glucan polymer which is amorphous in nature and acts as the matrix for accumulating antimicrobial compounds during pathogen attack. In this way, the delivery of these antimicrobial compounds becomes more targeted. Callose is deposited at higher levels in young leaves and seedlings

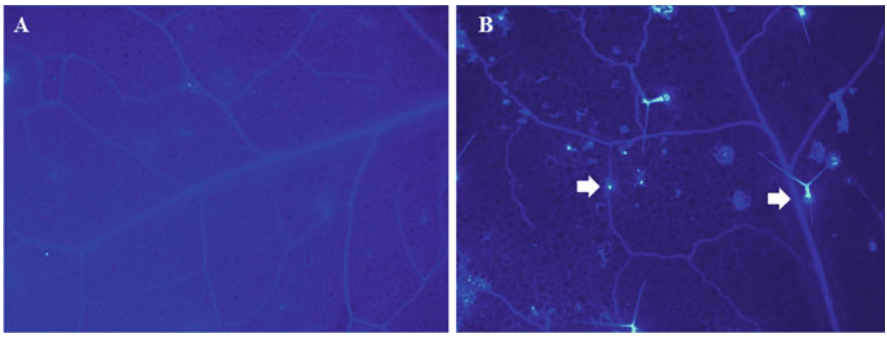

Fig. 4.1 Demonstration of callose in the wild type of *Arabidopsis thaliana*. (**a**) Shows the uninfected leaf, while (**b**) shows the leaf infected with *Pseudomonas* bacterium. An enhanced level of callose in the leaf tissue and leaf trichomes is shown with white arrows, and it is part of plant induced structural resistance cascade

compared to older tissues. Callose deposition is triggered by the specific molecular patterns of the pathogen. These patterns are called pathogen associated molecular patterns (PAMPs). A famous example of these patterns is the N terminal conserved 22 amino acid flagellin molecule from *Pseudomonas* bacterium (Fig. 4.1). In response to this bacterial PAMP, the model plant Arabidopsis influences the elevated level of production of callose in the adjacent cells. While in the case of *Xanthomonas*, a common pathogen of bacterial blights, this bacterium nullifies the effect of higher levels of callose by counteracting through the abundant production of Xanthan. Xanthan is a polysaccharide produced in the *Xanthomonas* spp. of the bacteria.

Some of the mutant lines like pmr4, which is for truncating synthesis of callose, were found to have normal papillae suggesting that the callose has minor or no role in the structural defense of the cell wall; instead, it strengthens the chemical defense of the plant. The pmr4 mutant sustained papillae formation at the sites of powdery mildew attack caused by *Blumeria graminis* f.sp. *hordei*, while the amount of callose was very low and only a minor increase in penetration frequency by the pathogen was observed, suggesting the chemical nature of callose in the defense rather than a structural barrier. Deposition of callose is considered important as it is considered as a marker and produced in response to pathogen PAMPs, while its synthesis during abiotic stress is also observed. The priming of plants with signaling hormones like salicylic acid or non-protein amino acid β-amino butyric acid (BABA) are strong inducers of the accomulation of the callose. BABA-induced priming of plants for callose deposition exploits the abscisic acid (ABA) dependent pathway in the model plant. Callose is also correlated with the amount of H_2O_2, which is important during abiotic stresses. So callose is multifaceted and augmented in response to PAMPs like Flg22 or polysaccharide chitosan, variations in growth conditions, and varying degrees of accumulation of H_2O_2, while treatment of ABA has both positive and negative impacts on the accumulation of callose (Ali et al. 2013).

4.8 Why Image Analysis Is Important

Pathogens with high or low inoculum are present in the majority of ecosystems. Under favorable environmental conditions, along with the availability of host crops, pathogens result in the onset of various diseases. Prevalence of such favorable conditions for extended periods often results in epidemics with compromised host plants' compromised resistance levels. Susceptible hosts are vulnerable and usually are considered the habitat of pathogens. They provide important support in the survival and persistent availability of pathogen inoculum for many upcoming cropping seasons. However, human interventions like the use of pesticides could help to manage such diseases and save agricultural produce for ever-increasing human populations and animal feed (Capote et al. 2012).

Pesticides are used after properly assessing disease in a particular field or area. Nonetheless, pesticides are not targeted, and healthy plants often get the dose of chemicals that have an economic cost and environmental concerns. From the perspective of early assessment of an event or wise calculation based on window-pane analysis, the algorithm is traditionally employed with disease outcomes. Climatic conditions are significant and directly correlate with disease severity in any geographical region. The model works on the correlation of weather events with the development of the disease. But this model does not explain the real disease development and environmental conditions. Instead, a scalar-on-function regression model with a binary output at every crop event is more reliable and demonstrates the real disease progress going on under these so-called favorable environmental conditions.

Plant diseases are assessed by observing visual symptoms and laboratory tests for confirmation. Such field observations are also converted into a quantifiable form as percent disease severity or disease index, as shown in Figs. 4.2 and 4.3. But the main

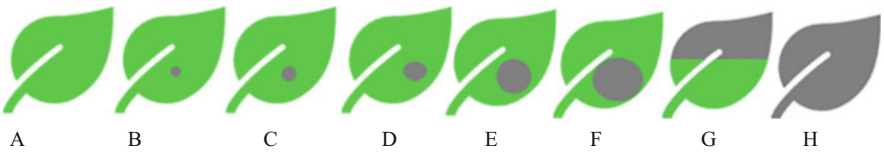

Fig. 4.2 Input disease indices for establishing disease severity scale ranging from healthy leaf shown in **a** and complete symptom development are displayed in **h**, whereas **b**–**g** show the varying intensity of symptoms with brown color

Fig. 4.3 Testing of disease severity scale in the field by conferring scoring from the established scorecard

problem is in the rating by individuals. Individuals vary in the ability to rate a particular disease because of different skills. Experienced and inexperienced raters differed in the ability to assess the same field having the disease (Bock et al. 2009). Such differences in the assessment are not reflected in the final conclusion of disease in a particular area or field, and decision-making could be compromised, which is entirely dependent on the individual data-driven campaign from the field.

During an inspection of the field for disease assessment, apparent symptoms like large lesions are easily estimated compared to small lesions in the same area. And the statistical analysis also verifies that error arises due to lesion number (Godoy et al. 1997). Similar studies on bean rust show that raters overestimated disease when they counted small lesions, which were higher in number in contrast to a few more extensive lesions with minute error (Hock et al. 1992). The plant tissue or organ and the size of the part under consideration also add in error during estimation, and severity could be different (Nita et al. 2003).

In the modern age, such disease rating is aided with online disease diagnosis using internet facilities. The revolution of smartphones with the internet is used for image analysis because of their fast computing ability, high-resolution cameras for taking images, and clear display. Computer vision, especially object recognition, has significantly improved in the past few years. It is widely used for many visualization-related problems in computer vision, including object classification per pre-trained instructions. The PASCAL VOC challenge for the recognition of images analyzing them based on a trained data set (Everingham et al. 2010), and more recently, the Large Scale Visual Recognition Challenge (ILSVRC) (Russakovsky et al. 2015) based on the ImageNet data set has made possible to analyze image-based input at a very high pace (Deng et al. 2009).

The algorithms are diverse, and selecting a suitable algorithm associated with image analysis is very important. All the related images of the disease under observation are collected. The input data is classified into different categories and attributes. The data set is divided into training and testing data sets with a ratio of 80–20%. Training of model is a challenging process and involves domain expert. The model is trained based on color (or wavelength), leaf segmentation, lesion size, lesion number, and leaf area (Mohanty et al. 2016). Once the model is trained on the data set of images, then the model is tested or subjected to transfer learning for the remaining data in the next phase. The training phase could be played with changing input attributes. After the training phase, the accuracy of the model is checked. Several experiments and epochs are important in the training phase. Epochs are the number of training iterations in which a particular neural network has completed a full pass of the training phase (Shah et al. 2019). The number of epochs is important to the well-converged learning of the model. Once the model is trained and tested, it is subjected to an unknown data set that will be infected with varying intensity of symptoms (Lin et al. 2013). Based on previous training, the model will classify the data based on attributes already selected during training. This trained model could be used in the form of a cell phone app. After providing the input image, the model will analyze and classify the image, and a particular disease condition associated with that image could be determined. The algorithms are trained for big data, and

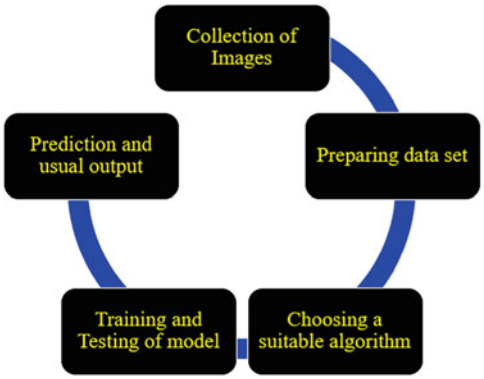

Fig. 4.4 Training, testing, and application phases of a model for disease plant image analysis

thousands of images from a disease crop field could be classified in a short time, and a targeted decision could be easily taken (Fig. 4.4).

4.9 Conclusion

The modern approach for image analysis is more sensitive, reliable, and fast, and a big data set can be analyzed in a very short time. Such interpretations help to make the decision. Ultimately, it would help to save resources by providing correct information. Such information on the diseased patch of the field will help to make the targeted decisions. It could also be a suitable replacement for biochemical tests, which are time-consuming.

Model performance entirely depends on the training and testing data sets. If similar symptoms appear due to abiotic stresses like heat or drought, the model will not differentiate them unless trained for such attempts. Furthermore, model availability in the form of mobile applications could further help in the fast disease diagnosis on farms.

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Hyperspectral Imaging Through Spatial and Spectral Sensors for Phytopathometry

5

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Abstract

The indexing of plant diseases in quantification plays a crucial role in precision agriculture. Identifying plant diseases with symptomology is the traditional and most commonly observed practice in plant pathology. However, visual observation involves human errors and time consumption. Diagnosis of diseases and their severity in the plant are being recorded through disease rating scales, and innovation in these techniques is the need of time. Hyperspectral sensors and imaging techniques provide precision in detection and involve efficient data analysis at different space and times. This chapter provides an overview of hyperspectral sensors and imaging technologies used to assess plant–pathogen interaction and their implications for plant disease management.

Keywords

Precision agriculture · Disease quantification · Hyperspectral imaging · Disease management

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5.1 Introduction

Phytopathometry is a fundamental and important part of plant pathology as epidemiology and disease management are dependent on it (Bock et al. 2020). Phytopathometry is the assessment of plant disease detection, identification, and quantification. The methods like color thresholding digital imaging have been employed for the accuracy in the percent area of the leaf affected by the disease (Goncalves et al. 2021). The pathogen's interruption of the plant's physiology results in modified reflectance from the surface, which can be determined by image analysis software for disease detection (Barbedo 2016; Ul Haq et al. 2020). The well-known image analysis software are Assess, ImageJ, Quant, and custom-made (Karisto et al. 2017). There is an advancement in quick and accurate disease detection by using artificial intelligence algorithms accomplished by a convolutional neural network (CNN) for semantic segmentation (Esgario et al. 2020).

Hyperspectral imaging (HSI) is used to simultaneously acquire spatial and spectral data from single cells and macroscopic objects (Gowen et al. 2015). The reflectance at visible and infrared wavelengths provides information about leaf pigments and host plant physiology, respectively (Sankaran et al. 2010). Remote sensing helps quick diagnosis of disease and accurate management decisions (Weiss et al. 2020). Remote sensing deals with acquiring knowledge about an object without physical contact from the ground, aerial, or satellite sources (Gogoi et al. 2018). Biophysical and biochemical features mainly describe the spectral characteristics of plantations. In the visible field (0.4–0.7 μm), the key light-absorbing pigments are chlorophyll a and b, carotenoids, xanthophylls, and polyphenols (Sahoo et al. 2015). RGB (Red, Green, Blue) image technique was used for predicting the disease outbreak; however, it has limitations under natural fields due to lighting and shade, which can easily be used in a greenhouse (Sugiura et al. 2016).

The spectral signatures of plant diseases that depict a particular developmental stage during pathogenesis were used to construct a spectral library. Machine learning analysis was used for detecting and quantifying diseases from hyperspectral images (Bohnenkamp et al. 2021). Thomas et al. (2017) have described host-microbe association through hyperspectral image analysis. The reflectance magnitude has an important role in detecting and quantifying the spatial and temporal progress of the host–microbe relationship. Mahlein (2010) explored leaf sugar beet diseases in the canopy and leaves using spectral signatures from hyperspectral sensors. The instrument having self-radiation is regarded as an active sensor, whereas the passive system operates with solar radiation (Schellber et al. 2008).

Hyperspectral remote sensing assesses biotic and abiotic stresses from leaf reflectance based upon spectral regions. Leaf pigments are dominant in the visible area of 400–700 nm, whereas the dominancy of leaf water contents and structure of cell lies in the near-infrared NIR (700–1000 nm) and short-wave infrared (SWIR, 1000–2500 nm) regions, respectively (Clevers et al. 2004). Changes in reflectance are attributed to modifications in foliage morphology and physiology. The reflectance of older and diseased plants was lower in the near-infrared band than in red (Li et al. 2005). Slonecker (2011) recommends a spectroradiometer to perform

in vitro and in vivo experiments. Hand-held spectroradiometers are used to monitor alterations in the spectral reflectance of plants. Hyperspectral devices enable immediate and detached identification (Kuska and Mahlein 2018). Thomas et al. (2018) described that hyperspectral devices are important in plant disease measurement. Innovative ways diagnose biotic stresses in plant robots, artificial intelligence, and satellites (Zheng et al. 2021). Artificial intelligence and machine learning are helpful in the physiological studies of plants (Walter et al. 2015). Big data is obtained from sensors using artificial intelligence and machine learning, which reduces the difference between theory and practical implications of hyperspectral science (Tardieu et al. 2017). The modern techniques increase the precision in diagnosis and reduce the chances for human error (Lobos et al. 2017).

Mahlein et al. (2019) used various sensors to diagnose head blight disease in vitro; the temperature of diseased plants differed from healthy ones, ensured through infrared thermography. Similarly, there was low photosynthesis in infected foliage, as depicted by chlorophyll fluorescence. Abdulridha et al. (2019a, b) used a hyperspectral (400–1000 nm) imaging system to detect citrus canker in vitro through radial basis function (RBF) and k-nearest neighbor (KNN). Top plant imaging used a crewless aerial vehicle to detect cankers in the field. Mahlein et al. (2013a, b) determined the severity of different fungal diseases by using a spectroradiometer at 450–950 nm wavelength, and data was analyzed through the RELIEF-F algorithm. Zhang et al. (2012) developed multivariate linear regression (MLR) and partial least square regression (PLSR) for analyzing hyperspectral images to assess powdery mildew disease.

5.2 Terminology

Absolute error is calculated as the difference between recorded and original disease intensity.

Accuracy: This is the closeness of recorded and actual values.

Adequate sample size: It is related to the sampled object, e.g., field, plant, plant part required to maintain accuracy.

Color transformation: Alteration in color to distinguish the patterns of an image.

Convolutional neural network (CNN): CNN works on a mathematical principal and is used to analyze digital images based upon pixel data.

Deep learning: It is used to predict on the basis of modern techniques and statistics.

Geometric three-dimensional (3D) sensor: 3D sensors measure the geometric surface of objects in space, to determine size and growth of plants.

Hue, saturation, and intensity (HIS) color model: Hue defines color in 0° to 360°; saturation white light (0–1), and intensity white to black (0–1).

Hyperspectral imaging: Captures images at different wavelengths and pixel wise spectrum is obtained for further analysis.

Image analysis: Use of digital images to detect, quantify, and identify the disease.

Light detection and ranging (LiDAR): Obtaining accurate 3D information through pulsed laser.

Machine learning: Use of artificial intelligence (AI) algorithms in disease detection and identification.
Reflectance: It is the ratio of reflected light to the incident light to an object.
Remote sensing: It is the phenomenon of taking the image of an object from a distance and then analyzing it through different software.
Sensor data fusion: A collection of data from multiple sensors to detect plant diseases.
Spectral signature: Severity of reflectance on electromagnetic spectrum for a particular plant condition.
An unmanned aerial vehicle: It is controlled without a pilot.
Visible (VIS) spectrum: Electromagnetic spectrum visible to the human eye, corresponding to wavelengths between 380 and 750 nm.

5.3 Visual Estimation of Plant Disease Incidence and Severity

Epidemics of plant diseases threaten food safety and security by lowering production quality and quantity in agriculture and forestry (Strange and Scott 2005; Savary et al. 2012, 2017). Visual information of plant disease incidences and their severities are the pre-requisites for estimating the crop losses, conducting disease surveys, marking the threshold levels, understanding the plant disease epidemiology, assessing the fungicides, and formulating the management strategies (Bock et al. 2020). A plant's disease symptoms may alter in shape, size, and color. The factor that is frequently most significant or interesting in a given experimental circumstance is disease severity (Paul et al. 2005). Visual estimation is the process of rating the severity of symptoms as the eye observes them. According to Madden et al. (2007), disease severity is the percent unit area of infected plants compared to healthy ones, whereas disease incidence is the ratio of infected units of plants in a specific population to healthy ones.

5.3.1 From the Observation to Remote Sensing

Remote sensing is an innovative and effective tool in the field of patho-phytometry. Based on remote sensing, a sensor or gadget evaluates the amount of sickness or stress signal directly or indirectly (Nilsson 1995; Bock et al. 2010). The capture of the image through a non-VIS spectral range can also be used to estimate the severity of the condition. The methods are different in efficiency regarding quantifying the severity of infection (Mahlein et al. 2012, 2018; Kuska and Mahlein 2018). These systems employ wavelengths in the band of 250–2500 nm, and the calculations are exclusively dependent on the perception of wavelengths. No one system covers the complete range; thus, just a portion of it is often used, typically the near-infrared (NIR) and infrared (IR) bands. To calculate the percentage of infected tissue, raters must be able to distinguish between asymptomatic and symptomatic tissue. The number of observed pixels matched with already known characteristic pixels that

reflect a healthy state compared to a diseased state, which is detected using a variety of statistical processes, is the basis for measurement in VIS spectrum image analysis. The distinctive wavelengths connected to the sick condition are measured by HSI and MSI devices. Study of image acquisition compared to visual estimations is although complex but benefits (Mahlein 2016).

Scaling of disease is an essential factor in selecting an evaluation method due to disease severity information at various spatial scales. Additionally, to meet the demands of "phenomics" in plant breeding, the severity evaluation is a prerequisite to aid the data on a genomics scale and provide accurate and fast assessments (Mutka and Bart 2015; Ul Haq and Ijaz 2020). In the phenomics age, high throughput is a crucial factor that affects development and effective resource use. The creation of optical sensors supports disease detection, severity, and categorization in a non-invasive manner. These technologies are already used in plant phenotyping and precision agriculture for resistance breeding (Fiorani and Schurr 2013; Kruse et al. 2014; Stewart et al. 2016; Mahlein et al. 2018).

5.3.2 Quantification of Disease Severity and Importance

The data used in remote sensing depict actual values. A study or assessment is considered accurate if it is near the "gold standard" or true value (Bock et al. 2010, 2016). It must consider how closely an estimate corresponds to the actual value when determining disease severity or measuring accuracy (Madden et al. 2007). Two types of bias: systematic bias (based on the size of the real number); constant bias (based on the overall tendency for estimation).

There are two types of reliability: intra-rater (or method) reliability and inter-rater (or method) reliability. Accuracy can hinder research and waste resources and has an adverse effect on grower income. To properly study treatment effects, comprehend yield loss correlations, interpret surveys, and rate germplasm phenotypes, accurate measures or estimates of severity are crucial. Additionally, the necessity to spray can arise from using severity data as a decision threshold or for illness predictions (or not). Different circumstances may call for various degrees of accuracy. Disease evaluation can lead to a false negative, also known as type II error, as shown by many empirical and simulation-based research (Chiang et al. 2014, 2017a, b). Although this hasn't been discovered in studies on disease evaluation, a false positive (type I error) could be just as harmful. Precise measurements or calculations will reduce these two inaccuracies.

Disease severity has been evaluated using nominal, ordinal, and ratio scales. Brief descriptions of disease like "No," "Mild," "Moderate," and "Severe" are used as the basis for nominal scales, and the symbols are "−" for no disease or healthy, "+," for mild "++," for moderate, and "+++" for severe. Interval scales, among the primary scales, are not present because of lacking real zero.

Nominal scales are arbitrary and can change depending on who is rating them and when. Statistical techniques based on rank or frequency might be used to analyze the data. The term "quantitative ordinal scale" Normative scales (quantitative and

qualitative) refers to numerical intervals from 0% to 100%, which is still unclear. These have been previously described as quantitative ordinal, category, and interval scales (Nutter Jr and Esker 2006; Hartung and Piepho 2007; Chiang et al. 2014; Bock et al. 2016).

Qualitative ordinal scales help measure the severity of various diseases without easily quantifiable symptoms. This group may include a variety of viruses, various systemic diseases, and root diseases, such as cassava mosaic disease and citrus huanglongbing (Hahn et al. 1980; Gottwald et al. 2007). These rank statistics are based on distinct reports of different expressions of symptoms and almost probably non-linear symptom development. Qualitative ordinal scales can also be examined using non-parametric statistics appropriate for various experimental designs and function distribution, or they can be studied using parametric statistics (Shah and Madden 2004; Fu et al. 2012). Equal or unequal intervals may be present in quantitative ordinal scales (Horsfall and Barratt 1945; Hunter and Roberts 1978).

Measurement scales: Many diseases allow for weight measurement using measuring scales. Quantitative ordinal scales can be analyzed using a proportional odds model, conversion of mid-point, or by first doing parametric analysis on the percent interval (Chiang et al. 2019). A disease severity index (DSI) can be created using the frequency of ordinal scores (Chester 1950). Actual severity and a severity index may or may not be related, but they differ fundamentally and shouldn't be used interchangeably. DSI may be particularly prone to overdose when using the above formula if intermediate values in the measurement class are ignored, according to a recent study by Chiang et al. (2017a, b). A standard scale used to measure severity visually is the % scale (Gent et al. 2018; Bock and Chiang 2019; Hamada et al. 2019; Xu et al. 2019).

5.4 Digital Imaging and Hyperspectral Imaging

It will be helpful to first think about what a common, non-hyperspectral digital image involves to understand hyperspectral technology. Central light waves, measured in nanometers (nm), are approximately 475 nm in blue light, 520 nm in the green light, and 650 nm in red light. Red, green, and blue are the three-wide wavelength bands that make up a color image. Each of the three types of cones in our eyes has a specific color range and is sensitive to the electromagnetic spectrum's blue, green, and red portions. The cones are either stimulated powerfully or weakly depending on the light wavelengths used to excite them. By combining the data from the three various types of cones, our brain creates a color image. The pixel in the digital image captures the integrated intensity of the blue, green, or red part of the light spectrum, depending on the filter in front of the pixel to mimic the sensitivity of the receptors.

A hyperspectral device can capture broad light. A small portion of the electromagnetic spectrum, from 400 to 700 nm, corresponds to the color visible to the human eye. The wavelength range from ultraviolet (UV) (starting at 250 nm) to short infrared waves (SWIR, 2500 nm) is commonly used in hyperspectral plants. To cover most of the spectrum, some sensors include a small range of standard cameras,

such as optical and near-infrared (VIS-NIR, 400–1300 nm), SWIR (1300–2500 nm), or UV (250–400 nm). An actual multispectral image usually has additional bands, which may be the sample light emanating from the infrared part of the spectrum and—the light intensity of more than 700 nm. Hyperspectral images usually consist of hundreds of small wavelengths within the spectrum. With enough space adjustment, the path produces a dense, rich color database with hundreds of data points (pixels) per leaf. The wide range of visible and infrared light sources seems to be very effective in analyzing flora and fauna. This spectrum can detect changes in mesophyll cell structure (700–1300 nm) and leaf color (400–700 nm); however, wavelengths (1300–2500 nm) are required to detect effects on plant moisture (Peñuelas and Filella 1998). However, low drought pressure often does not have a sufficient effect that can be detected (Satterwhite and Henley 1990). Excessive drying, for example, may interfere with the formation of the mesophyll leaf, leading to changes in near-infrared light.

Hyperspectral imaging spectrometers use various hardware techniques; therefore, there are a few ways in which the image is obtained. Push brooms, filter wheels, and flexible liquid crystal filters are just a few examples of effective devices (Fong and Wachman 2008). Another use of a push-up vacuum cleaner involves transmitting incoming light through a convex grid (or prism) that separates it into waves of a certain length. A light sensor records this distortion (similar to a standard digital camera). The three components comprise the vacuum cleaner's device: camera, spectrometer, and lens. This technology simultaneously records the entire color spectrum and one area line in the image. Then, the camera or object is removed to capture the next line, thus converting it into a line scanner, and the final image is created when the full scan is complete. Other push-broom variants include a summary mode, in which a complete picture is taken with a single shot.

5.4.1 Disease Indices in Use

Before the widespread discovery of hyperspectral imaging devices, researchers who sought to measure outcomes based on color knowledge used multispectral, or hyperspectral, point-source devices (such as spectroradiometers, which do not produce a local image) to obtain color information. Generally, targeting and clicking ratings are not provided by hyperspectral instruments. Instead, the user has a great responsibility to create the recording process. Large data sets from acquisition must be analyzed to obtain significant results. Considering just a few of the wavelengths, looking at changes in all conditions in the sensitive areas previously described in the spectrum is a logical and direct approach to such large databases.

The normalized difference vegetation index (NDVI), which measures the general health status of crops (Lasaponara and Masini 2007), is one of the most well-known and widely utilized metrics. For instance, *Eurygaster integriceps* Put. (Hemiptera: Scutelleridae), the Sunn pest/cereal pest that stresses wheat has been detected in wheat using NDVI (Genc et al. 2008). The majority of indices are highly specialized

and only perform effectively with the data sets for which they were intended (Verrelst et al. 2006).

Disease-centric studies focus on creating disease indices for detecting and quantifying specific diseases (Verrelst et al. 2006). One study used the leaf rust disease severity index (LRDSI) with an 87–91% accuracy in detecting the leaf rust (*Puccinia triticina*) in Wheat (Ashourloo et al. 2014) but to our knowledge, it has not been widely tested. Another commonly used method is finding a difference in the sudden increase in visibility on the red/near-infrared border. As chlorophyll absorbs most wavelengths up to 700 nm, the material is not clearly visible in this range but is strongly infrared, resulting in significant changes in the spectral (alternate) reaction of green vegetation in this area (from about 720 nm). According to Cho, the red edge can be extracted or found using various techniques (Cho and Skidmore 2006).

5.4.2 Complete Spectrum-Based Classification

The goal of classification techniques is to separate the data into various unique classes. They come from a group of machine learning or statistical methods. One such method is quadratic discriminant analysis (QDA), which differentiates classes by the covariance matrix. A study of avocado plants investigated Laurel wilt fungus (*Raffaelea lauricola*) using garden plants and a glass house. QDA method used. QDA had an accuracy rate of 94% (Sankaran et al. 2012). Of course, alternate techniques can be used at each step of the analysis pipeline. For instance, a decision tree approach (a machine learning technique) has been utilized in place of QDA and has achieved 95% accuracy (Sankaran et al. 2012). The key is selecting the best data analysis strategy and guaranteeing a large, high-quality dataset. An increasing number of categorization and prediction algorithms are using such machine learning techniques. Machine learning techniques train algorithms using a training data set to analyze and forecast outcomes from new, unforeseen data. An illustration of such a method is the multi-layer perceptron (MLP).

MLP neural networks use primary networks to map output input data. This method is based on the biological knowledge of networks that control how neurons interact with each other. The activation function and adjustable weights are used to update the input area and produce the desired output (using training data). This method requires prior knowledge (training data); therefore, it is wrong to use this procedure if the "disease spectra" are unknown. With the third separation method, the basic pattern or data modification is assessed by looking at spectral fingerprints using alternatives. It is necessary to use "slide" before inserting the output because the output from the second (and higher) system usually does not care for light variability (Tsai and Philpot 1998). However, they are susceptible to noise, which hyperspectral data frequently experiences. To produce a smoother signal, smoothing uses various forms of averaging to lessen the difference between the brightness of individual pixels and those of nearby pixels. Savitzky–Golay and Gaussian filters are two types of smoothing. Savitzky–Golay proposed a way to reduce jitter in noisy data by inserting local polynomials into a subset of data input and smoothing signal

by polynomial testing in one place (Schafer 2011). Gaussian filtering lowers noise by applying a Gaussian-weighted kernel to average the spectral data and concentrate on the center information.

5.4.3 Use of Hyperspectral Data

When several plants are photographed over several days, hyperspectral data is huge in size. A single plant scan may easily take up one GB or more. The process will take much longer if the entire spectrum is analyzed than if only a few wavelengths are chosen. The data does contain much information, some of which may be important. If your camera records data in 800 spectral bands, you must decide whether you need all 800 bands or whether a combination of data at 400, 200, or other bands will suffice. This compares to compressing RGB images in a JPEG-like format. This compression reduces file sizes at the expense of permanently erasing image information (especially color). Few spectral bands are stored, leading to smaller files and a simpler process of data analysis at the expense of color markers that may be significant. Spectrographs acquired using three different system setups are calibrated and characterized by (Polder et al. 2003). The experiments examine the various noise kinds and the signal-to-noise ratio. By estimating the resolution, the spectral range, and the number of pixels, the studies also show that, to some extent, binning can take place without information loss.

5.5 Early Detection of Citrus and Solanaceae Plant Diseases Using Remote Sensing

5.5.1 Remote Sensing in Citrus Diseases: Case Study

Citrus is a significant crop from a commercial point of view. The citrus orchards are present in all the tropical and sub-tropical areas of the world. Among the devastating diseases of citrus, bacterial canker (CBC) caused by *Xanthomonas citri* is the most threatening and is widely distributed (Terentev et al. 2022; Schubert et al. 2001; Das 2003). Another critical disease is Huanglongbing (HLB), caused by *Candidatus liberibacter* spp., and is transmitted via insect vectors from one place to place and plant to plant (Deng and Tang 1996). The main effect of these organisms is that they interfere with the nutrient supply of the plants, which can ultimately cause the plants' death. Polymerase chain reaction (PCR) and other nucleic acid-based techniques are the leading and efficient sources of diagnosis of HLB worldwide. To date, ecofriendly and effective method to control and manage the disease is challenging. Only measures are present to slow down or reduce further disease infestation by destroying the trees infected with HLB (Jagoueix et al. 1996; Fan et al. 2009; Mishra et al. 2012).

Remote sensing, with its two types, active and passive, is one of the latest and most influential and non-destructive techniques for the early detection associated

with managing diseases (Terentev et al. 2022). Many articles have been published claiming to elaborate on the partial control measures related to HLB using the hyperspectral remote sensing (HRS) method. Principal component analysis (PCA) is the widely used technique in HRS (Sankaran et al. 2011). Li et al. (2012) used the red edge in the orange orchard field and laboratory experiments on two orange cultivars, "Valencia" and "Hamlin" orange, to find significant differences between healthy and infected HLB trees. The indoor database showed a higher level of accuracy than the external citrus fruit database (approximately 95% compared to 90%) due to better natural conditions and more citrus samples.

Overall, the accuracy of the visual aspect ratio of the data ranged from 43% to 95%. When using a multispectral camera instead of a hyperspectral, Kumar et al. (2012) found very accurate results (87% vs. 80% accuracy). In both multispectral and hyperspectral images, the MTMF (Sorted filter) and (SAM spectral angle) SAM method was most effective (Kumar et al. 2012). With the help of (at least squares—vector support machine) LS-SVM. Weng et al. (2018) described healthy subdivision models infected with HLB (invisible and marked) and nutrient-dense orange leaves of local orange varieties, namely, Unshiu and Ponkan. These models had 90.2% accuracy, 96.0%, and 92.6% of the coolest, hottest, and all-time seasons, respectively. The authors have demonstrated the effectiveness of combining citrus carbohydrate metabolism and hyperspectral reflection imaging to identify HLB in various cultivars and growing seasons. The HLB of the Ponkan cultivar was obtained using a split model designed for the Satsuma cultivar data set with a modulation model, with an average accuracy rate of 93.5% and a low number of false negatives. In a study of non-destructive early detection and classification of citrus HLB disease, Deng et al. (2019) have successfully detected HLB early. By using the PLS-DA method and testing it with an exit-without-verification strategy, the study could provide three models for early detection and HLB disease planning (Deng et al. 2020; Lan et al. 2019, 2020; Mei et al. 2014). Smooth and precise methods have a very good impression on the third model built using Savitzky's pre-processed spectral data from Golay; it had a grade accuracy with a total accuracy of 96.4% and a predictable accuracy of at least 92% in five types of leaf samples.

5.5.2 Remote Sensing in Solanaceae Plant Diseases: Case Study

One of the most popular food crops in greenhouses and outdoors is the Solanaceae family, which also contains tomatoes, potatoes, tobacco, peppers, and other plants. These crops are susceptible to various diseases that really can result in significant yield losses, including early and late blight (Foolad et al. 2008; Akino et al. 2013), several viruses (Roselló et al. 1996; Karasev and Gray 2013), bacterial and target spot (Hardwick 2006), among others. Due to the substantial economic costs associated with these diseases, it is crucial to identify them early to implement prompt and effective control strategies. The publications in this part that discuss the early diagnosis of such disorders utilizing HRS are highlighted. Zhang et al.

(2003) detected PLB disease under field conditions through hyperspectral imaging and elaborated on the most affected patches.

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Fluorescent Imaging System-Based Plant Phenotyping for Disease Recognition

6

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Abstract

Plant phenotyping is an emerging field that associates genomics with plant agronomy, physiology, and eco-physiology. Plant phenotyping has become a promising field in plant breeding research. Various phenotyping platforms have been developed that involve imaging technologies and robotics in recent years. However, these platforms have limited potential restricted to only a few plant species. To resolve this issue, various imaging methods are employed for the data collection, quantitative studies of very complex traits of plant development, their yield and production rate, and their response to various environmental stress. Main imaging techniques are visible imaging, imaging spectroscopy, 3D imaging, and, most importantly, fluorescence imaging. This chapter briefly reviews the fluorescent imaging techniques for plant phenotyping and their applications in this field.

Keywords

Imaging techniques · Imaging · Imaging spectroscopy · 3D imaging · Fluorescence imaging · Plant phenotyping

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6.1 Introduction

6.1.1 Plant Phenotyping

Plant phenotyping is the evaluation of genetically diverse traits like growth, reproduction, endurance, resistance, morphology, metabolism, ecology, and productivity, as well as the primary assessment of individual quantitative factors, which serve as the foundation for more complex features (Mir et al. 2019). The plant phenotype comprises complicated characteristics, including root morphology (Clark et al. 2011), biomass (Menzel et al. 2009), leaf characteristics (Jansen et al. 2009), fruit qualities (Brewer et al. 2006), photosynthetic efficiency (Bauriegel et al. 2011), and biotic as well as abiotic stress responses (Singh et al. 2013; Naeem et al. 2020; Anjum et al. 2020; Ul Haq et al. 2020). Recent assessments of phenotypic traits for resistance to disease in genetic improvement depend mostly on professional morphological observations, which are time-consuming and can lead to biases among specialists and researchers. Plant phenotyping has gained importance as a topic of study in disease diagnosis (Phenodays 2014). Plant phenotyping aims to assess complex features, including development, production, and environmental stress tolerance, with a high degree of certainty and clarity at all developmental stages (Fiorani and Schurr 2013). High-throughput phenotyping programs have recently been used in culture rooms and glass houses. Such systems measure plant effectiveness and development using robotics, accurate environmental control, and imaging techniques (hardware and software). Nevertheless, these platforms are only appropriate for a small number of species, such as Arabidopsis rosette plants (Granier et al. 2006) and some cereal crops (Yang et al. 2013). It is necessary to develop and establish such platforms that allow phenotypical assessment of multiple crop species.

Numerous plant phenotyping platforms have been established to study complex plant characteristics associated with the development ability to adapt to biotic or abiotic stress conditions; however, the potential to acquire high-throughput phenotypic data has become a hindrance in the plant genomics study. In recent years, scientists have undertaken substantial experimentation and research on high-throughput image-based phenotyping techniques, such as visible light imaging, fluorescence imaging, thermal imaging, spectrum imaging, stereo imaging, and tomographic imaging (Zhang and Zhang 2018).

Early and precise identification and analysis of plant disorders are critical for crop yield and a reduction in qualitative and quantitative losses. RGB imaging, multi- and hyperspectral sensors, thermography, and chlorophyll fluorescence are examples of optical techniques that have demonstrated their ability in automatically generated detection systems for detecting plant diseases at the initial stages of disease outbreaks. 3D imaging has recently been included as visual analysis, providing extra details and information on crop plants' health. Many systems, from proximal to remote sensing, are available for dynamical observation of individual plant organs and vast field areas. Innovative data analysis methods lead to accurate and precise plant disease detection. Sensor-based approaches support molecular approaches for

plant disease detection and disease management (Haq and Ijaz 2020). Agriculture and crop phenotyping are two highly relevant fields for the applications of sensor-based analysis (Mahlein 2016). The colors of grains or seeds, plants leave, canopies, fruits, roots, and the size of seeds, shapes, numbers, and textures can all be recorded directly utilizing two- or three-dimensional imaging systems. Using light, thermal, fluorescence, phantom, and 3D imaging systems, pathogenic characteristics associated with plants and pathologic characteristics linked to insects can be assessed directly or indirectly (Araus and Cairns 2014).

6.2 Fluorescent Imaging System and Its Principle

The chlorophyll compound is a fluorescent element in all plant species. As the chloroplasts are subjected to light, chlorophyll re-emits a part of the radiation that was absorbed, resulting in fluorescence. Fluorescence is light produced in a plant whenever smaller wavelength emissions are absorbed.

Furthermore, a source of actinic illumination combined with short, saturated blue fluorescence can be utilized to evaluate photo-assimilation effectiveness, non-photochemical quenching, and other physiological characteristics in the plants. The fluorescence imaging system is comprised of a fluorescence signal excitation system, collection of the fluorescence signal set element, and indicator (Gorbe and Calatayud 2012).

UV irradiation (between 340 and 360 nm) generates dual forms of luminescence, i.e., red and far-red fluorescence as well as blue-green fluorescence, which is the base of the multicolored fluorescence imaging system (Gorbe and Calatayud 2012). The method captures fluorescence signals in four spectral bands: blue, green, red, and infrared. The origin of fluorescence signals in the blue-green region is ferulic acid, primarily present in the cell wall, while the origin of signals in the red-infrared is chlorophyll-*a* (Kumar et al. 2015). Luminescence emission alterations and proportions amongst emissions in different regions, such as F440/F690 and F440/F740, can be utilized as stress indicators, with the F690/F740 ratio being a valuable measure of chloroplast contents (Li et al. 2014).

6.3 Florescent Image Processing Techniques

For the analysis of plant phenotypic characteristics, first, evaluate and interpret the images generated by various imaging technologies before studying the phenotypic traits of plants. Image coding, transformation, compression, enhancement, restoration, segmentation, description, and classification are standard image processing processes. Image segmentation is the most significant pre-processing operation, followed by feature extraction. Following that, data analysis is used to determine the target plant attributes.

6.3.1 Image Segmentation

The excellence of the final picture analysis output is determined by picture segmentation. As a result, it is among the most crucial and, in many circumstances, the most difficult phases in image processing (Cheng et al. 2001). Thresholding is considered one of the traditional and widely used approaches. The thresholding algorithms divide pixels into groups based on similarities, considering that particular objects in a picture comprise pixels with comparable attributes. These limits can be calculated empirically using training examples (supervised approach) which can also be calculated based on the picture's information.

In most cases, an image is separated into portions with its threshold. A histogram is frequently created using pixel characteristics. The thresholds that separate the parts can then be calculated using various statistical methods. A typical method for detecting irregular patches is edge detection. In the segmentation technique, the margins of items of interest in a picture are assumed to correspond to sharp changes in pixel characteristics. Kernel-based filters depend on computed discrete derivatives and are used in several edge detection algorithms (Ghanbari and Atangana 2020).

6.3.2 Feature Extraction

Feature extraction is a computer vision idea that recovers higher-level characteristic data from pictures. As color attributes are generally specific to particular objects or scenes, color attributes are a highly prevalent feature form. Color qualities are also more resilient than other visual aspects. As a result, a more concise depiction of color in imagery or a general area might be quite useful. Shades of colors are portrayed in images by utilizing a certain color model. The RGB, hue-saturation-intensity, and hue-saturation-value color models are all often used. The histogram, which assesses the worldwide distribution of colors in such styles, is the most frequent presentation for spectral features (Lenk et al. 2007).

Texture features describe the factors that measure the 3D designs of gray-scale or color differences inside an illustration. The most frequently applied texture classes are (1) statistical, (2) transformational, and (3) model-based. Quality traits are frequently employed in plant phenotyping to distinguish different items, such as leaves, trunks, buds, plant seeds, flowers, and fruits (Materka and Strzelecki 1998).

6.3.3 Analysis of Data

The fundamental challenge in achieving particular plant phenotyping objectives is data analysis. Spectral signs have already been established to associate biophysical and biochemical plant factors with plant development (Blackburn 2007). To determine healthy and unhealthy plants, spectral information can be translated into several forms, such as variation spectra, ratio spectra, and derived spectra (Pietrzykowski et al. 2006). Spectral vegetation indices (SVIs) have been created

and commonly used to examine, assess, and visualize temporal and spatial fluctuations in foliage. SVIs reduce the data dimension for disease identification and its discrimination by calculating ratios between spectral bands. As pigment quantity indicates the physiology of plants, certain color SVIs are expected to effectively detect fungal infection severity (Delalieux et al. 2007).

Classification is a process of attributing spectral signs to specific classes or programs and distinguishing these fractions from each other. Primary elements assessment, also known as principal component analysis (PCA), spectral angle mapper (SAM), and machine learning methods are the very conventional techniques applied for categorization (Rumpf et al. 2010). Most remote sensing classification algorithms can be used to identify disease-induced spectral variations because infection outbreaks and symptoms manifestation produce temporal and geographical differences in crop reflectivity. Rumpf et al. (2010) used support vector machines to effectively discriminate against sugar beet foliar infections in the pre-symptomatic stage (Rumpf et al. 2010). Data mining approaches have been demonstrated to be more effective at distinguishing between stressful events and disorders (Moshou et al. 2004). It is possible to distinguish between nutritious and canker-infection dented fruit of citrus with a spectral-based algorithm (Qin et al. 2009).

Multivariate approaches, including partial least squares (PLS), are the most extensively employed method for processing hyperspectral pictures. PLS constructs numerical patterns to plot hyperspectral information. Both spectrum and trait data are integrated into the standards for measurement; PLS-based models frequently exceed other models. When models have been built and verified, these could be used in routine analyses to anticipate phenotypic variables from outer sets of data and then combined with ecological and genotypic information to help breeders make choices (Cabrera-Bosquet et al. 2012). However, when using multivariate approaches, employing a large set of phenotypical and genotypical information is important to form the standard and prevent incorrect results (Kumar et al. 2015).

6.4 Application of Fluorescent Imaging

Fluorescent imaging is commonly employed to assess plant physiological and pathological characteristics. Fluorescent imaging could be employed to assess plant condition and activity of photosynthesis. It could also be utilized to analyze plant respiration, determine the impact of pest and insect-resistant genes in plants, and track plant pathogenic diseases. Moreover, it could also be used to identify the response of plants to biotic and abiotic factors and stresses like salinity stress before any developmental defects (Pérez-Bueno et al. 2016). Fluorescent imaging can detect physiological variations in tissues that are not detectable with a visible light camera. Researchers can use this characteristic to spot early symptoms of various biotic and abiotic stress and take preventative measures (Xu et al. 2021). Fluorescence imaging, as one of the helpful instruments in phenotyping approaches, can offer information about plant photosynthesis and facilitate the selection of helpful plant features in plant adaptation to biotic and abiotic stimuli (Cen et al. 2018). It is a

promising method for analyzing photosynthetic activity at the intracellular, foliar, and whole-plant levels, enabling plant phenotyping (Pérez-Bueno et al. 2016). Fluorescence imaging can assess photosynthesis and detect initial trauma reactions to abiotic and biotic variables before a drop in development and can be quantified to monitor the effects of plant diseases (Balachandran et al. 1997; Baker 2008; Chaerle et al. 2007).

Fluorescence imaging is a quick way to see if a plant's metabolism and growth are improving or deteriorating. Barbagallo et al. (2003) used descriptions of the parameters of fluorescence *Fv*/*Fm* for the growth of seedlings in a 96-well plate, and it was proposed that fluorescence imaging is a technique for studying stress-inducing compounds to test genotypes.

For example, Swarbrick et al. (2006) explored barley-infested leaves' endurance reaction with *Blumeria graminis* through fluorescence imaging. Photosynthesis was gradually inhibited over the entire leaf during a susceptible contact. Chaerle et al. (2007) monitored sugar beet lines that varied in their sensitivity to *Cercospora beticola* disease (Babar et al. 2021) and discovered that changes in fluorescence intensity were documented among susceptible plant and resistant plants using chlorophyll fluorescence imaging. Bürling et al. (2010) used fluorescence imaging to investigate variations in the amount of cultivar of wheat resistant to *Puccinia triticina*. He found that in PSII, they may be distinguished by employing the quantum produce pattern of non-regulated energy degeneracy.

Furthermore, high-throughput screening is done for photosynthetic variations and distinguishing mutations through diverse photosynthetic pigment components. It has been proposed as probable usage of chlorophyll fluorescence imaging for QTLs for development-linked characters. Approaches that utilize laser-induced fluorescence transients (Meroni et al. 2009) are being investigated to obtain quantities at the whole plant and its canopy level.

6.5 Disadvantages

Fluorescence imaging is a potent tool for resolving the three-dimensional variability of foliage photosynthesis activity; in addition, it has been employed in a variety of plant detection applications, including the initial finding of any symptoms caused by pathogenic agents. Visual observation cannot identify some metrics, such as growth disparities (Barbagallo et al. 2003).

The usage of phenotyping at a large scale and to build a standard approach for fluorescence imaging, picture dispensation, robustness, reproducibility, and data analysis tools is required. In the fluorescence imaging studies, most studies are restricted to single leave or seedlings of model crops. Furthermore, the supremacy necessities of fluorescence imaging may be prohibitive for applications of the field phenotyping (Bouvrais et al. 2010; Bibi et al. 2011; Murphy and Davidson 2002).

Choosing the correct fluorescent tint or dye is vital, as some dyes can create substantial fluctuations in the host membrane or may cause investigational errors, leading to inaccurate information and erroneous analysis (Bouvrais et al. 2010).

Long-term exposure to fluorescent light can cause color removal and reduce fluorescence intensity. When employing fluorescence microscopy, selecting fluorescent dye and making other new membrane probes are important aspects (Klymchenko et al. 2009).

For disease diagnosis, pathogen morphology is studied under microscopes. Pathogen isolation on selected artificial media is possible using microbiological methods, and pathogen identification and detection can be accomplished using molecular and serological approaches (Ijaz and Khan 2009; Ijaz 2011; Ul Haq et al. 2019). Plant protection services, as well as research and industrial development, utilize these methods for pathogen identification. In recent years, DNA-based strategies and serological techniques have revolutionized pathogen detection and disease identification (Bock et al. 2010; Martinelli et al. 2015; Ward et al. 2004; Ijaz et al. 2019; Ul Haq and Ijaz 2019). Since 1999, producers and quarantine inspectors have had access to highly specific and rapid testing methods. To analyze and identify plant diseases, tests like these could be used immediately on a site of fields, in a glasshouse, or throughout the production chain. For example, *Phytophthora infestans*, *Ralstonia solanacearum*, *Erwinia amylovora*, and some viruses such as Pepino mosaic virus, Tomato mosaic virus, Potato virus Y, and Potato virus X can all be detected using a variant of ELISA (Danks and Barker 2000). Strains of pathogen or their isolates that vary in virulence or are tolerant to a particular fungicide can be detected using molecular and serological approaches (Ijaz et al. 2020). However, as the quantity of pathogenic biomass might not always be proportionate to the point of observable disease symptoms, the degree of pathogenic growth in an infected plant is not always associated with disease intensity (Nutter Jr 2001).

Training professionals have undergone extensive research and analysis for visual observation and analysis. Conventionally, plant diseases are detected by observing plant disease symptoms or visible pathogen symptoms. However, visual estimation is always influenced by a person's understanding and expertise and could be modified by sequential fluctuation (Bock et al. 2008; Newton and Hackett 1994; Nutter Jr 2001).

The challenges in the diagnosis and detection of plant infections were highlighted by Riker and Riker in 1936. They presented an outline of the advantages and limitations of existing approaches before concluding, "We need improved diagnostic procedures; any one of the methods among cannot be considered standardized. They are only effective while good quality processes are established" (Riker and Riker 1936; Steddom et al. 2005). These long and time-taking approaches require qualified persons with sophisticated diagnostic and disease detection skills and are consequently prone to person handling errors.

6.6 Prospects

Future-oriented plant safety involves new and innovative methods to resolve forthcoming issues and trends in agricultural yield that require more precision than ever owing to consumer oversight, as Riker and Riker said in 1936. New sensor-based

approaches for discovering, diagnosing, and quantifying plant diseases have recently been discovered (Hillnhütter et al. 2010; Mahlein et al. 2012a, b; Sankaran et al. 2010; West et al. 2003). The latest computerized techniques with extraordinary sensitivity and specifications are required to improve disease identification beyond visual estimate processes.

Sensors that detect reflectivity, plant temperature, or any emitted fluorescence are currently the most promising techniques (Chaerle and Van Der Straeten 2000; Mahlein et al. 2012a, b; Sankaran et al. 2010; West et al. 2003). These devices measure the visual characteristics of plants in various parts of the electromagnetic spectra and can use data that is not apparent to the naked eye. As the disease can induce changes in the color of the tissue, shape of the leaf, rate of transpiration, canopy structure, and density of the plant, these devices allow the identification of initial variations and alterations in the physiology of plant due to various to biotic and abiotic stresses (West et al. 2003).

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Concept and Application of Infrared Thermography for Plant Disease Measurement

7

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Abstract

Fungal diseases cause significant losses in crop production, yields, and quality worldwide. Early and precise diagnosis and measurement of plant diseases are critical for crop production and agricultural yield reduction, both qualitatively and quantitatively. Early diagnosis and measurement of plant disease in the field allow farmers to treat infected regions more quickly and precisely anticipate output losses. Traditional detection approaches focus on pest scouting and visual inspection, often detecting the disease after the ideal control period has ended. Early detection and measurement will assist in avoiding disease spread to adjoining crops or fields, in addition to reducing losses of the individual producer. Hyperspectral and multispectral visualization techniques can assist in visualizing fields for plant disease protection by using diagnostic disease symptoms, including alteration in leaf color, structure, and moisture level. Infrared thermography is a thermal imaging technology currently used to detect plants stressed by biotic and abiotic factors. Temperature fluctuations have already been linked to

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pathogen attacks in various instances. In this way, infrared thermography in plant pathology allows for early and quick disease measurement. This technique is a non-destructive, non-invasive, and non-contact approach to determining the thermal characteristics of any material of concern, and it may be used in a wide range of applications where heat is produced or lost over time.

Keywords

Disease diagnosis · Hyperspectral and multispectral visualization · Thermal imaging

7.1 Introduction

Early diagnosis of plant-pathogen infection is critical for effective disease management and prevention (Chaerle and Van der Straeten 2000; Bauriegel et al. 2011; Lowe et al. 2017). Most plant diseases are caused by pathogenic fungi, which develop characteristic symptoms on plants as blight, cankers, lesions, galls, and other apparent disease symptoms, i.e., rots, wilts, blotches, and damping-off within a few weeks of disease development. These signs and symptoms entail variations in the plant's color or function due to the plant–pathogen interaction. Conidia of Erysiphales are apparent indicators of a pathogen (Takamatsu 2013). However, some pathogens develop disease symptoms at late infection stages; for instance, when powdery mildew infects rose plants, it does not produce any disease symptoms (e.g., red blister-like lesions) or pathogen signs on the upper leaf surface at early disease development stages.

Similarly, the mycelium of white powdery disease caused by *Podosphaera pannosa* occurs on plant leaves at many later stages of disease development as in powdery mildew (Coyier 1983; Linde and Shishkoff 2003). Consequently, consultants, professionals, and farmers must have exceptional observational abilities to detect disease early, which is not always attainable. Furthermore, the duration from host plant infection to the onset of disease characteristic symptoms (incubation period) is not constant and is highly reliant on the pathogen, host plant species, and environment factors (temperature, air, and relative humidity (Tomerlin and Jones 1983; Roger et al. 1999; Fraser et al. 2004). The diagnosis and management of disease symptoms and the development of direct and indirect approaches to identify the causal organism depend on characteristic disease symptoms (Nutter Jr. 2001; Bock et al. 2010).

In this regard, the lack of apparent symptoms quickly after a pathogen attack and the difficulties in estimating the incubation period create substantial hurdles to early infection identification and management, preventing disease progression and transmission. Techniques like digital photography and infrared thermography are already being applied to collect information about whole plant health or individual plant parts to measure plant diseases as soon as feasible after pathogen invasion (Xu et al. 2006). Image sensors for plant disease measurement, including digital image sensors

such as RGB, multispectral, hyperspectral, and infrared thermography, have been reviewed (Lowe et al. 2017).

It has been found that RGB and hyperspectral photography are preferred for diagnosing particular plant diseases, such as distinct forms of mildew produced by *Blumeria graminis* f. sp. *tritici*, causing wheat powdery mildew (Lowe et al. 2017). Hyperspectral cameras, on the other hand, are necessary to produce high-precision findings. Compared to RGB cameras, multispectral and hyperspectral cameras have more accurate images for several plant diseases because multispectral and hyperspectral pictures utilize visible light and a broad spectrum of electromagnetic waves (Nagasubramanian et al. 2019). The application determines whether to use multispectral or hyperspectral photographic methods. Hyperspectral and multispectral sensors are highly costly. Additionally, lighting circumstances might cause inaccuracies or false information in multispectral, hyperspectral, and RGB digital images concerning plant health (Kwan 2019). This raises significant challenges to the practical application of the above methodologies in field and greenhouse plants, implying that hyperspectral remote sensors might be a potential option for some of the measurement issues outlined above (Kwan 2019).

Plant temperature is measured using infrared thermography. This is linked to the status of plant water and variations in transpiration, which a variety of factors can cause, including early plant disease development (Jones 1999a; Oerke et al. 2006; Mahlein 2016; Belfiore et al. 2019). The data linking variations in leaf temperature associated with plant transpiration, which was driven by several plant-pathogen infections, have been found (Jones 1999a; Vagelas et al. 2021). Foliar plant diseases, including leaf spots, significantly impact plant organs' transpiration rate and water movement (Mahlein 2016). Many various processes, including mechanical or enzymatic damage to leaf cell membranes, stomatal opening stimulation mediated by pathogen-produced toxins, and plant vessel clogging by fungal hyphae, can result in water loss or deficiency, and hence a change in leaf temperature (Mendgen et al. 1996; Wang et al. 2015; Kwan 2019). Due to the collapse of mesophyll cells induced by pathogen toxins, image processing indicated a local reduction in temperature. Tobacco mosaic virus infection in the same plant leaves, on the other hand, was identified early that use the same procedures by a local reduction in temperature.

Thermography technologies were used to visualize *Cercospora beticola* infections on sugar beet leaves at an early infection stage before the development of the plant damage (Chaerle et al. 2004, 2006). Infrared thermography also signifies that yeasts, mushrooms, and molds all kept their temperatures lower than their surrounding temperature (Cordero et al. 2020). As a result, temperature variations on the plant leaves surface may indicate the presence of an infection.

Infrared sensors provide low-cost thermography access, ideally suited to observing variability in the plant due to stress (Lee et al. 1993; Jones 1999a; Vagelas et al. 2021). Infrared cameras with long thermal waves, referred to as thermal cameras, are calibrated sensors that can capture emitted rays in the thermal range (8–14 m) and produce pictures showing temperature readings per pixel. As a result, temperature data might be substituted with thermal pictures to assess plant physiological conditions in a short time (Pineda et al. 2020). Because leaf temperature appears

to be a highly effective indication of plant physiological condition, plant phenotyping depending on imaging methods has become a widely available and significant technique in agriculture (Li et al. 2014). Both abiotic and biotic stresses, such as drought, high salinity stress, insect pest aggressiveness, and microorganism invasions, cause changes in leaf temperature (Jones 2004; Prashar et al. 2013; Khanal et al. 2017).

Thermography is frequently used in conjunction with other imaging remote sensors. Image processing and data analysis tools are used to improve precision agricultural approaches. Even though thermography is an incredibly useful tool for detecting early biotic and abiotic damage on host plants before symptoms develop. When reading thermograms concerning temperature, there are a few things to keep in mind, such as water adequacy or plant scarcity. This is because the surface temperature of the host plant is determined mainly by environmental elements, including temperature, air, and relative humidity (Pineda et al. 2020). The main objective of this chapter is to demonstrate the general concept of thermography and the application and efficacy of thermal infrared cameras (consuming less cost than other remote sensors) to measure plant diseases.

7.2 Background

In addition to regulating water supply and general metabolic activity by external factors, pathogens may impact plant cells' stomatal and cuticular conductance, leading to substantial changes in leaf temperature. Digital infrared thermography can identify management regions in plant disease control since leaf temperature may be recorded digitally and with excellent spatial resolution. To apply site-specific, targeted, or on-demand fungicides in integrated plant disease management, it is critical to notice changes in plants or canopies related to minor disease severity during the initial phases of disease epidemiology. The detection of ethylene, which has been linked to pathogen cellular injury (Boller 1983), and optical techniques for analyzing the reflection and fluorescence features of plants, both being related to the photosynthetic mechanism, are some strategies (Coops et al. 2003; Laudien et al. 2004; Franke et al. 2005).

Thermography provides a quantitative examination of spatial and temporal physiological data on the condition of a plant (Jones 2004). In plant pathology, infrared thermography is applied to investigate spatiotemporal heterogeneity of stomatal conductance (Jones 1999a; Omasa and Takayama 2003; Prytz et al. 2003), to plan irrigation (Gebhardt 1990; Jones 1999b), to measure ice-nucleation or temperature tolerance in host plants, to analyze pathogen–plant interaction by screening temperature patterns on the surface of plant leaves, (Wisniewski et al. 1997; Yang et al. 2003; Oerke et al. 2006), and to monitor for mutants with modified stomatal regulation (Chaerle et al. 1999; Oerke et al. 2006).

Remote sensors must be sensitive to physical problems related to fungal invasion and infection arising from pathogen invasion and tissue colonization for detecting, identifying, and quantifying plant diseases and related pathogens remotely.

Unlike weeds, which can be identified and recognized digitally in crops early after emergence due to their macroscopic size and shape (Gerhards and Christensen 2003), microbes that cause diseases in plants can only be identified by their impact on plant cells; characteristic symptoms generally occur just after latent colonization of plant cells. Previous research has demonstrated that remote infrared thermography may be utilized to identify *Pseudoperonospora cubensis* causing cucumber downy mildew before symptoms appear (Lindenthal et al. 2005; Oerke et al. 2005). Under a controlled environment, maximum temperature difference within a plant leaf or a canopy becomes appropriate to differentiate between affected or unaffected tissue.

7.3 Precision Agriculture for Managing Plant Diseases

Plant diseases are a major stumbling block to agricultural yields worldwide. Modern farming policies strive to reduce the application of fertilizers and pesticides by targeting and integrating cultural management strategies to control weeds, insect pests, and pathogens (Maloy 2005). Precision agriculture is based on the establishment of technology, including thermography techniques allowing the detection and mapping of obstacles in agricultural fields (Cerovic et al. 1999; Mulla 2013; Baron et al. 2012, 2016). Such techniques can be utilized to assess the impact of pressure on plant metabolism. As a result, thermography has evolved into non-destructive solid tools that are now indispensable; they deliver critical information to decide and for the proper timing to apply the treatments. (Usha and Singh 2013; Li et al. 2014; Mahlein 2016).

7.4 Thermography

The conversion of images created by thermal wavelength radiation radiated by colder elements to make such wavelength images visible is the primary purpose of thermography (Williams 2009). The thermography term can also be applied to systems in which no visual images are created. However, a thermal image is captured and processed entirely electronically to measure or quantify certain specific characteristics or detect the presence of an entity or a pathogen. Thermography is appropriate because of four main features (Davis and Lettington 1988):

(i) It is completely a passive technology that does not need an external light source to assist day and night process and integrate detection.
(ii) It may identify hot or cold spots and distinct emissivity zones.
(iii) Infrared radiation penetrates fog and haze more quickly than visible light, facilitating the recognition of optically obscured objects.
(iv) It is a real-time digital sensing approach.

The field of thermography has advanced rapidly in recent years. Agriculture, soil moisture investigations, industries, pharmaceutical, veterinary, and military applications utilize thermography (Vadivambal and Jayas 2011). Infrared thermography techniques have been used in a broad range of areas due to the availability of the current commercial system. Through this technology, the temperature of any substance surface may be traced with high precision; however, while monitoring outside surface temperature, various elements such as heat, wind, fog, and rain impact the potential of thermographic images (Holst 2000; Hashim et al. 2020).

7.4.1 Principles of Infrared Thermography

The principle of infrared thermography is dependent on the fact that all objects above absolute zero generate infrared radiation, particularly at −273 °C temperature (Prakash 2000). The infrared is an unseen band of light on the visible electromagnetic spectrum with wavelengths ranging from 0.75 to 100 m. Near-infrared (0.75–2.5 m), shorter-infrared (1.4–3 m), medium-infrared (13–8 m), and long-wave infrared (>8 m), and extreme infrared (15–100 m) regions all constitute the infrared region (Tsai and Hamblin 2017).

A long-wave infrared system of infrared thermography is appropriate at room temperature, while the medium-wave infrared system is useful at higher temperatures (i.e., 400 °C). Short-wave infrared devices are more effective at scattering reflected radiation than emitted radiation (Tsai and Hamblin 2017).

Transmission windows having a mostly translucent environment to infrared light are the base to divide the infrared spectrum. Thermographic tools can monitor infrared rays from short to long wavelengths. The emissivity and temperature of a material influence the emission of its radiation. Thermal sensors use infrared detectors to convert the infrared radiation emitted by a target material into an electric signal. It emerges as a colored or monochromatic thermal photograph with a color shift representing the thermal fluctuation of material (O'Donnell et al. 2014). The fundamental advantage of thermography is that it is a non-destructive, contactless, and simple instrument for detecting temperature distribution over a short period.

The quantity of emitted radiations by a body is determined by its temperature T and emissivity. Generally, the emissivity of a perfectly white object is about 0 and 1 for a perfectly black thing.

The released infrared radiations quantified by an infrared camera may be transformed into an electric signal and presented as a monochromatic or colored picture using an infrared thermal camera sensor.

Based on infrared energy emitted by the object, the temperature of the object's surface can be measured.

$$W = \sigma T_s^4 = \frac{2\pi^5 k^4}{15\, c^2 h^3} \times T^4 \tag{7.1}$$

Here W denotes emitted flux energy per unit area (W/m^2), T_s denotes the temperature of the material surface (K), and Stefan–Boltzmann constant ($= 5.67 \times 10^{-8}$ W/m^2 K^4) is denoted by σ; however, c represents the light speed in a vacuum while k and h denote Boltzmann's constant and Planck's constant, respectively.

The emissivity of substances ε is requisite for real substances. For perfect black bodies, the valid equation is as given below:

$$W_r = \varepsilon \sigma T_r{}^4 \tag{7.2}$$

Here the emitted radiation energy is denoted by W_r, which is the sign of the temperature of body T_r that emits radiation energy. So, the basic concept of infrared thermography is the requirement of W_r visualization to apprehend the emitted radiation energy (Gowen et al. 2010; Lee et al. 2018).

7.4.1.1 Passive Infrared Thermography

Passive infrared thermography is easier as compared to active infrared thermography. It entails just the detection of infrared thermal visualization without the application of an external source of energy to the body during analysis. The thermal disparity in a picture is only visible if temperature variations exist concerning ambient temperature due to thermal emissions from the surrounding environment. The examination is carried out employing passive mode in most infrared imaging techniques. The thermal energy radiated by objects is detected by the infrared camera, which simply monitors phenomena. This suggests that the temperature trends detected are entirely attributable to actual temperature variances. As a result, the surface thermal parameters of an object are determined by thermal imaging collected in the passive mode (Lee et al. 2018)

7.4.1.2 Active Infrared Thermography

Active infrared thermography, on the other hand, entails exposing the object to thermal energy before cooling and heating it. The variances in thermal resistance caused by flaws cause anomalous thermal dispersion during the thermal transfer process, which occurs during the flow of internal heat in the body. This mode of infrared thermography can be utilized to determine the flaws under the sub-surface (Skala et al. 2016; Lee et al. 2018). Two distinct active infrared thermography methods are discussed here.

7.4.1.2.1 Lock-in Mode Thermography

By introducing a sinusoidal fluctuating thermal energy, the lock-in mode creates a thermal wave and movement of heat within the specimen. This thermography mode is responsive to local surface influences in the phase image (Meola et al. 2006). A halogen lamp or a laser light for a sinusoidally fluctuating light source will be employed as the source of heat. A halogen lamp or a laser light can be used as the sinusoidally fluctuating light source. The penetration intensity of the emitted thermal energy is determined by the switching frequency used. The frequency domain is

utilized to analyze the thermal properties, which are then used to determine the type and depth of the flaw.

7.4.1.2.2 Pulse Mode Thermography

The thermal sensor can track the thermal retardation of the sample from the reflected thermal flow when a pulse is applied by taking sequential photos at regular intervals. The acquired visual depicts the reaction of the object's parallel region to each pulse. The heat flow rate within the body is changed by the areas with various characteristics, including flaws on the object, and the thermal characteristics of the body differ from those surrounding it. To disrupt the thermal equilibrium in the object, a pulse mode thermograph provides a short-term energy pulse to the object. This mode of infrared thermography is a quick qualitative assessment approach; however, background interference and non-cracked emissivity may be severely impacted by heat response properties (Sakagami and Kubo 2002).

7.4.2 Application of Infrared Thermography

Plant diseases have impeded crop production in agriculture and resulted in significant economic losses. For example, global crop losses due to biotic disease are predicted to result in a $220 billion revenue loss per year (Faostat 2019). Farmers should be able to decrease the prevalence of infections in its development. Research has revealed that thermography may be used to analyze and monitor geographical and temporal patterns in plant diseases at different phases of disease progression. Infrared thermography can identify and quantify local crop infections caused by pathogens before the initiation of crop damage. Thermal sensing can have considerable implications for highly sensitive infection prediction models to spatial and temporal variations in the host temperature. The physiology of infected plant tissue, including transpiration rate, photosynthetic alteration, salicylic acid accumulation, the conductance of stomata, and death of plant cells, is modified during pathogen infection (Xu et al. 2006). Through digital optical sensor techniques, foliar plant diseases can be diagnosed and quantified directly. The thermal infrared technology has been considered an efficient tool for visualization, detection, or quantification of plant disease before the development of visible symptoms (Ishimwe et al. 2014).

Infrared thermography is associated with microclimate in plant stands and variations in transpiration; it is used to measure plant temperature driven by initial plant pathogenic infections (Jones et al. 2002; Oerke et al. 2006; Lenthe et al. 2007; Ul Haq and Ijaz 2020). Infrared thermographic cameras can perceive infrared energy radiated in the thermal infrared range between 8 and 12 μm. This radiation may then be seen in false color pictures with each image pixel, including the temperature data of the target material. Infrared thermography may be applied in plant research at many temporal and geographical dimensions, including airborne and small-scale applications. However, it is often subject to environmental factors such as sunlight, ambient temperature, rainfall, or wind speed. There is a strong link between leaf temperature and plant transpiration, which various diseases impact differently (Jones

1992; Jones et al. 2002). While several foliar pathogens, including rusts and leaf spots, cause only localized changes in the affected area, root pathogens (such as *Rhizoctonia solani* or *Pythium* spp.) or systemic infections (such as *Fusarium* spp.) frequently affect the rate of transpiration and water flow throughout the whole plant or its organs. Plant-virus associations in tobacco and *Cercospora beticola* causing leaf spots in sugar beet have been linked to local temperature fluctuations caused by pathogen infection or plant defensive mechanisms (Chaerle et al. 2004). Nilsson (1991) proved practical applications by showing a strong link between the severity of several root and leaf diseases in diverse crops (wheat, barley, oat, potatoes, sugar beet, etc.). The variability between and among leaves can be used to analyze infrared thermography efficiently. An important marker for the emergence of plant disease is the mean temperature difference between individual crop stands, plants, and leaves.

Infrared thermography has been applied in many agriculture sections, including monitoring greenhouse and nurseries, programming irrigation, estimating crop yield, detecting plant disease, assessing fruit maturity, and detecting bruising in vegetables and fruits. Infrared thermography has been applied many times in plant pathology and is a very effective technique for detecting and quantifying plant diseases early.

7.5 Disease Detection and Measurement

The physical condition of infected host tissue is transformed during plant–pathogen interaction, including alterations in transpiration, photosynthesis, the buildup of salicylic acid (SA), stomatal conductance, and even host cell death (Xu et al. 2006). Advanced optical sensor technologies may directly monitor plant foliar disease. Powdery mildew of barley, infected cucumber leaves, and wheat yellow rust were all identified through hyperspectral reflectance sensors (Olivier et al. 2003) and (Moshou et al. 2004). However, because plants are delicate to physiological disorders related to fungal infection, digital infrared thermography has the efficiency in detecting and measuring management regions in controlling plant diseases and affiliated pathogens via high spatial resolution. Additionally, foliar diseases usually disturb the transpiration of infected plants (Oerke et al. 2006).

While analyzing the varying temperature allocation between the infected tomato leaves by tobacco mosaic virus strain-TMV-U1 and the healthy or non-infected leaves of different tomato species through digital infrared thermography in addition to microscopic observations (Xu et al. 2006) revealed the reduction in leaf temperature approximately 0.5–1.3 °C lower for the infected leaves compared with the non-infected leaves. However, the maximum temperature difference reduced as the infection progressed due to a rise in leaf senescence and leaf transpiration at all phases of scab formation. Maximum temperature difference may be utilized to distinguish between infected and non-infected leaves and measure the disease. Infrared thermography can also detect and measure plant pathogens before they develop apparent symptoms or signs.

7.5.1 Disease Detection and Measurement in Grapevines

The potential of infrared thermography to detect and measure pathogen (*Plasmopara viticola*) in both the irrigated and non-irrigated grapevines (*Vitis vinifera* L. cv. Riesling) has been explored. The study was conducted by the following four in vivo treatments: (i) irrigated grapevines without inoculation, (ii) irrigated grapevines with inoculation, (iii) non-irrigated grapevines without inoculation, (iv) non-irrigated grapevines with inoculation. Thermography has revealed that pathogen activity increased leaf temperature at the inoculation site in irrigated grapevines, but non-irrigated grapevines exhibited lower temperatures at the infection sites. To detect and quantify the temperature variation between diseased and healthy leaves, thermography is a simple way of delivering information to diagnose biotic pressure on the plant before characteristic symptom development, i.e., a manifestation of apparent necrosis on infected plant leaves (Stoll et al. 2008; Kim et al. 2014).

7.5.2 Disease Detection and Measurement in Apple

Plant pathogenic fungi may affect plant tissue's cuticular and stomatal conductance, leading to substantial changes in leaf temperature. Apple scab disease is caused by *Venturia inaequalis* colonizing leaves under the plant cuticle. Infrared thermography is one of the most appropriate approaches to detect and measure the development of pathogen (V. inaequalis) on apple plants. The pathogen isolates N5, SID, and HS1 each differ in virulence and were reproduced in apple plants by applying spore suspensions onto the leaves of apple plants and incubating them at 100% relative humidity for 48 h. Before incubation, the inoculum is sprayed on apple leaves @ 1–6 droplets (20 μl) of spore suspension through a commercial hand sprayer having about 1 cm distance among drops. The inoculated apple leaves approve that with increased concentration of inoculum, the temperature intensity decreases within the plant leaves while the degree of affected leaf region enhances. After 5 days of inoculation, symptomatic leaf tissue temperature profiles viably display the differences in characteristic lesion size and pathogen intensity in tissue colonization. Additionally, the alteration in the water status of the infected plant might be detected or measured 1–3 days before the development of characteristic symptoms by fungal colonization (Oerke et al. 2011).

7.5.3 Disease Detection and Measurement in Rose Plant

The capacity of infrared thermography to detect early indicators of fungal infections on rose plants (Rosa hybrida L) was investigated. The best derived thermal properties with the greatest linguistic fence values were chosen after performing a set of features. After 2 days of inoculation, the best prediction scores for detecting and quantifying gray mold and powdery mildew before symptoms appeared were

80% and 69%, respectively (Stoll et al. 2008). The findings of this study demonstrated that it is possible to identify and quantify powdery mildew and gray-mold infections before they manifest symptoms.

7.5.4 Disease Detection and Measurement in Sweet Potatoes

Research on viral infection due to co-infection during the cultivation of sweet potatoes (*Ipomoea batatas* L) was conducted. Observing the morphological and physiological impacts of disease in sweet potatoes during 29 days, the application of infrared thermography and chlorophyll inflorescence in photosynthesis output within sweet potatoes has been assessed. The spread and integration of the virus in sweet potatoes were associated with the treatment alterations observed in infrared thermography and chlorophyll inflorescence technique. This study initially verified using infrared thermography and chlorophyll inflorescence techniques to determine the severity of infections caused by the SPCSV and SPFMV viruses concerning sweet potatoes (Wang et al. 2019).

7.5.5 Disease Detection and Measurement in Wheat

Thermography can be applied to discriminate between infected and uninfected wheat plants at six dpi, at least 4 days earlier than the appearance of apparent symptoms. This study has resulted in these two outcomes. The maximum temperature difference and the average temperature of uninfected wheat stalks have not altered appreciably during the monitored period. Moreover, the average temperature indicated a consistent decline, and the maximum temperature difference of the inoculated wheat gradually raised as the inoculation days passed. The findings revealed that infrared thermography is a reliable technology for quick and non-disruptive screening and quantification of wheat yellow rust that can also detect wheat stripe rust at the initial stages of invasion (Yao et al. 2018).

7.5.6 Disease Detection and Measurement in Peanut

The diagnosis of peanut leaf spots was made using thermography and spectroscopy techniques. Two sets of thermal evaluations were conducted, first relying on the complete canopy and second based on a single host plant. Infrared thermography measurements in the first set showed that the infected region had a greater radiance than the uninfected region. These thermal characteristics may be caused by the decreased root absorption potential in diseased plants, particularly noticeable at the warmest times when the plant needed more water. The second set of evaluations was performed in an individual plant that was observed for thermal action and precise infrared responses throughout the day. The information indicated that the infected plant was found to have a 2.2 °C greater temperature than the uninfected plants.

Before the leaves' evident necrosis, the temperature difference allowed differentiation between infected and uninfected plant leaves (Omran 2017).

7.5.7 Disease Detection and Measurement in Oil Palm

The effectiveness of thermography in identifying basal stem rot infected an oil palm (*Ganoderma boninense*) tree has been examined. Based on the infrared thermal visual examination, it was possible to distinguish between healthy and basal stem rot infected trees by extracting characteristics using the Principal Component Analysis (PCA) approach. Depending on trendline figures, it is possible to distinguish between non-infected and trees infected with basal stem rot. The Support Vector Machine is better than the k-nearest neighbor with higher precision, i.e., 89.2% and 84.4% precision percentage during training and assessing sessions. Thermography can be used to distinguish between basal stem rot infected and healthy trees. The technique can be used in further research to categorize various disease severity levels (Bejo et al. 2018).

7.5.8 Disease Detection and Measurement in Cucumber

Infrared thermography is a practical approach for predicting the pre-symptomatic influence of pathogens and disease on infected plants. (Oerke et al. 2006) Infrared thermography was applied (Oerke et al. 2005) to identify and measure *Pseudoperonospora cubensis* causing downy mildew of cucumber. Under a controlled environment, the maximum temperature difference within a target leaf helps distinguish between infected and non-infected plant tissue. Under un-controlled environments, the temperature of the leaf and rate of transpiration were similar for infected and non-infected leaves, but for infected plant tissues, transpiration rates varied greatly depending on disease symptoms.

7.5.9 Disease Detection and Measurement in Tea Plants

Classification of rapid thermography was investigated to diagnose diseases of tea plants. To boost detection accuracy and make the UAV last longer, a grayscale canopy visual scatter curve is created to construct a classifier that can instantly spot the disease visual. A coefficient of determination (R^2 of 0.97) is found between the lesion number algorithm and human observational counting, which is 2% more compared to the lacking algorithm classifier. This research provides recommendations for using aerial thermographic visuals to monitor the status of tea gardens (Yang et al. 2019).

7.6 Conclusion

The management of polycyclic plant diseases depends on the early diagnosis and measurement of pathogen infections. Conventional serological and nucleic acid tests were applied to identify plant diseases. However, new cutting-edge techniques, including digital remote sensing, different sensors, and precision agriculture, are used to quickly and effectively detect and measure plant diseases. In contrast to other remote sensing techniques, applying infrared thermography in plant pathology offers the benefits of sensitivity, high analysis speed, and convenient usage. In addition to early illness detection, this method creates a solid foundation for thorough disease analysis through computer programming skills.

This advanced technology makes it simple to measure and monitor the surface temperature of the infected plant, which makes it a suitable tool for (i) identifying plant disease, (ii) differentiating between active and inactive mycelium, and (iii) measuring the phase of plant disease invasion regarding temperature variation.

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Application of Biosensors in Plant Disease Detection

8

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Abstract

Over the past years, significant advancement has been seen in detecting plant diseases. Early plant disease detection has a significant role as it ensures the farmer takes important steps to save crops from failure. Biosensor technology is a remarkable advancement with many advantages like its sensitivity and less time for detection. This chapter discusses a brief history of biosensors, design, component and working principle of biosensors, appealing features, and various biosensors, while illustrative examples of these biosensors depict their role in plant disease detection. Moreover, the untamed potential of these biosensors in plant disease detection with certain limitations is briefly discussed. In the end, future prospective and opportunities for detection based on biosensors are discussed.

Keywords

Biosensors · Components of biosensors · Plant disease detection

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8.1 Introduction

Plant pathogens are a constant threat to crops as they cause numerous diseases and significant crop losses (Fletcher et al. 2006). Approximately, 83% of infectious diseases are due to fungi, 9% are due to viruses and phytoplasmas, and more than 7% of diseases are caused by bacteria (Khakimov et al. 2022). Almost 14.1% of the crop is lost due to pests and diseases (Kourous 2016), accounting for a $ 220 billion loss in annual agriculture trade (Agrios 2005).

There is plenty of evidence in the history of agricultural science that widespread disease outbreaks have resulted in disastrous outcomes. Plant diseases still inflict considerable harm to agriculture today, despite the availability of contemporary, more reliable techniques for preventing and protecting plants from disease outbreaks in a specific area. Plant pathogens are mainly managed by chemical control and agricultural practices such as crop rotation, disease-free seed use, resistant varieties, and seed treatment with fungicide (Sharma et al. 2011; Mancini and Romanazzi 2014). Fungicides effectively control plant pathogens (Davidson and Kimber 2007; Allayarov et al. 2019; Ul Haq et al. 2020). However, excessive and long-term usage of fungicides has resulted in fungicide resistance among fungal populations (Mamiev et al. 2020; Donoso and Valenzuela 2018). Delays in diagnosis and the inability to take preventative actions can result in significant crop losses and a drop in product quality. Thus, it is consequently critical to developing accurate, timely, sensitive, and rapid pathogen detection methods to apply optimum chemistries more effectively (Dyakov and Elansky 2019; Narayanasamy 2011). Timely detection of the pathogen will reduce the lavish use of chemicals, minimizing the pressure on the pathogen's population and hazardous environmental impact (Khakimov et al. 2022). Various biochemicals, molecular, and culture-dependent methods are developed to achieve this goal. However, these methods are inappropriate for detection because they lack accuracy, reliability, rapidity, and accuracy.

Consequently, biosensors for the early and rapid detection of various plant diseases are of significant interest. Moreover, there is a broad scope of innovative strategies based on biosensing technology for plant disease detection. In this chapter, we discuss various methods for the detection of plant disease with particular reference to biosensing technology and quote multiple types of biosensors with their specific characteristics and their role in plant disease detection. Moreover, limitations in using biosensor technology are also discussed.

8.2 Plant Disease Detection

Crop inspection through visual observations is the oldest traditional approach for disease and perhaps pathogen diagnosis. However, the process of inspection through traditional ways is so slow that the pathogen is likely to have established itself in host populations when the diagnosis is made. To overcome these problems, current efforts for disease diagnosis are being employed, which mainly focus on developing the techniques with earlier pathogen detection technologies having accurate, sensitive, and speedy detection processes.

Despite advancements in these technologies, these widely used methods have drawbacks, such as complex sample preparation steps, long diagnostic time, transporting samples from the field to specialized laboratories, and requiring trained professionals. Consequently, an in-field diagnostic procedure that is faster, more reliable, exceptionally sensitive, and accurate is required. The main qualities of optics or electrochemistry could be used to construct a "point of care technology." This method improves bioassays by allowing quick response time without requiring the person's interpretation skills and low-cost on-site experiments (Lau and Botella 2017; Cassedy et al. 2020).

8.2.1 Traditional Methods of Plant Disease Detection

Plant disease diagnosis is improving all the time. New methods and technologies are being developed and used to assure the accuracy and speed of diagnostics. Traditional diagnostic procedures, however, retain their importance and enable the fast, rapid, and accurate diagnosis of plant diseases using newly developed equipment and technology. This allows for precise and quick decision-making about controlling agricultural crop diseases. Plant pathology research employs various diagnostic techniques: visual inspection, microscopy, mass spectrophotometry, mycological diagnosis, biological diagnosis, molecular genetics identification, immunological diagnostics, and other methods are examples of these (Reich et al. 2013; Kamilov et al. 2020; Zuparov et al. 2020).

8.2.2 Modern Methods of Plant Disease Detection

The disease diagnosis based on the host plant's symptoms is not always accurate. Because many diseases have symptoms similar to physiological abnormalities induced by external stimuli, some pathogens produce asymptomatic or weakly identifiable symptoms in the early stages of development.

The long time it takes to diagnose diseases using indicator plants, as well as the necessity for specific isolation chambers and employing this method where pesticides are already being used, affects the accuracy of the diagnosis. Furthermore, the same symptom can be caused by various factors, i.e., bacteria, fungi, or non-infectious diseases. This makes diagnosing diseases and precisely identifying their pathogen challenging. As a result, it can be inferred that diagnosis of disease based on symptoms is not a good approach. The necessity to solve current issues in traditional plant disease diagnosis has led to new approaches for detecting and diagnosing pathogen presence in plant pathology. Several advanced approaches are employed in phytopathology to identify pathogens. Immunological diagnostics, mass spectrophotometry, molecular-genetic identification, and other techniques are among them (Ahmad et al. 2012; Fang and Ramasamy 2015).

Crop disease detection can be done using both direct and indirect methods. When a large number of samples are to be analyzed, molecular and modern serological approaches for direct disease detection are utilized for high-throughput analysis.

Plant pathogens are directly detected via these approaches, allowing for reliable pathogen identification. On the other hand, indirect approaches employ morphological changes, transpiration rate changes, temperature changes, and volatile organic compounds emitted by diseased plants to detect plant pathogens (Lukhmenev 2012).

8.3 What Are Biosensors?

A biosensor is an analytical instrument that uses a bio-element recognition component in close contact with the transducer to detect chemical or biochemical occurrences at the sensor surface qualitatively or quantitatively. Bio-element recognition component reacts with the analyte and transforms biochemical change into a signal, which is then processed before being displayed on the screen (Arnold and Meyerhoff 1988). A biosensor can employ any transduction mechanism, such as loop-mediated isothermal amplification, electrochemical, surface plasmon resonance, quartz crystal microbalance, fluorescence, and lateral flow techniques for the generation of plant biosensors (Lowe 1984; Choi et al. 2017; Le et al. 2017; Wong et al. 2017; Arora 2018). Few biosensing approaches for plant biosensing have been established despite the availability of a wide range of biomolecules to recognize the desired analyte.

8.3.1 History

The concept of a biosensor is not so old. Cremer in 1906 first time gave the concept of the biosensor. He found that the quantity of acid in an aqueous medium equals the electric potential developed between the medium (Nikhil et al. 2016). This cleared the way for Leland C. Clark, Jr., called the "father of biosensors," to build "real biosensors" in 1959. Clark made a sensor to detect glucose by using a glucose oxidase electrode which detects the oxygen or hydrogen peroxide in biological samples. Afterward, significant advancement has been seen in developing highly selective and sensitive biosensors (Clark Jr and Lyons 1962; Li and Lee 2020).

8.3.2 Components of Biosensors

Biosensors comprise three major components: biological sensing element, transducer or physicochemical detector, and a signal processing system (Malhotra et al. 2017). Biological sensing elements react with the target analyte to develop a signal. Important sensing elements include microbes, enzymes, antibodies, organelles, tissues, cell receptors, and nucleic acids.

The transducer modifies the signal formed by the reaction of the target analyte and biorecognition component into the detectible signal. Then the electrical signal is amplified by the processing system and delivered to the data processor, transforming it into a quantifiable signal such as a color change, digital display, or printout (Malik et al. 2013).

8.3.3 Design of Biosensor

A biosensor comprises two parts: transducer and biological sensor element. A biosensor is frequently used with signal processing equipment, including a printer or display screen (Korotkaya 2014).

8.3.4 Biological Receptor

The biological receptor is also called a sensor or detector element, which detects the presence or concentration of an analyte or substance. The receptor is a biological component with biochemical properties used to precisely detect the target analyte (Chaubey and Malhotra 2002). When interacting with a target analyte, the biological receptor gives a signal in pH, light, charge, heat, or mass change. The receptor must be stable, specific, and immobile. The biological receptor must recognize the target analyte in the sample to be tested selectively. The biological receptor controls the device's sensitivity by producing a signal that the transducer evaluates (Paddle 1996). A tissue, microbe, enzyme, antibody, nucleic acid, organelle, or cell receptor, for example, can be a component. Catalytic and non-catalytic receptors can be separated into two types (Castillo et al. 2004). Devices for continuous monitoring of chemicals at the concentration of millimolar or micromolar use the catalytic group of biological receptors. Microbes, enzymes, and tissues are examples of them. The non-catalytic group is used primarily in biosensors monitoring analytes such as medicines, hormones, and toxins, typically in very low quantities. These are disposable devices that are used once before being discarded. Antibodies, nucleic acids, antigens, and other receptors are examples (Pearson et al. 2000).

8.3.5 Transducer

A transducer is the biosensor's second most important component. A transducer, in general, is a material that can transform one type of energy into another (Bhalla et al. 2016). A transducer in a biosensor converts the physiochemical signal obtained from the biological receptor. The interaction between biological receptor and target analyte produces a measurable signal that can be optical, piezoelectrical, electrochemical, and so on. The transducer senses and quantifies changes in the biological receptor–analyte interaction (Thévenot et al. 2001). An example of a transducer is a pH sensor in a glucose biosensor. Glucose oxidase acts as a biological receptor and is used to bind glucose which is then converted into gluconic acid in oxygen's presence. The transducer detected a change in pH and transformed into a voltage change (Bojorge Ramírez et al. 2009). While designing a transducer, reaction time, analyte concentration range, specificity to the target analyte and suitability for its application should be considered. A suitable transducer should be extremely analyte-specific and quite sensitive to quickly give readings at least analyte concentration (Sethi 1994).

8.3.6 Working of Biosensor

A biosensor operates on the principle of signal transduction. All biosensor components are combined and intended to translate biological changes into an electrical signal and, eventually, a measured output. Biosensors, to put it another way, can analyze the chemical by converting its biological reaction into a detectable signal(Soleymani and Li 2017).

The target analyte in the sample to be tested first reacts with the biological receptor in a specific way, causing a physiological change. The physicochemical parameters of the transducer near the biological receptor are also altered, resulting in the transducer's electronic characteristics, which are then translated into a detectable electrical signal (Njagi and Kagwanja 2011). Depending on the type of biological receptor, the signal can either be a current or voltage. If the output of the transducer is in the form of current, it will be converted into voltage.

Furthermore, because the output voltage is often low, the signal processing unit must perform further modifications, processing, and amplification through many filters. Finally, the signal processing unit's output should equal the quantity of target being measured. (Ali et al. 2017).

8.4 Characteristics of Biosensors

While designing a biosensor, certain features and characteristics must be considered by keeping the nature of the applications of biosensors. These variables determine a biosensor's performance and utility.

8.4.1 Sensitivity

This is thought to be the most crucial feature. The sensitivity can be defined as the connection between the alteration in the concentration of the analyte and the strength of the signal emitted by the transducer. A biosensor should, in theory, produce a signal when minor changes occur in the target analyte's concentration. Biosensors can detect analytes at concentrations of ng/ml or fg/ml, depending on the application. This is typically required for medical and environmental monitoring applications (Wang et al. 2012).

8.4.2 Selectivity

In the presence of other molecules or chemicals, this refers to the biosensor's ability to bind and react solely to the intended analyte. A false-positive result occurs when a signal or reaction is formed when it reacts with non-target analyte. This is typical in low-selectivity biosensors, which fail in clinical applications (Polatoğlu et al. 2020).

8.4.3 Stability

The biosensor's stability is critical, especially for biosensors that are utilized for continuous monitoring. This characteristic affects the biosensor device's ability to maintain its function over time in the face of disruptions caused by external sources. The external sources could be temperature, relative humidity, and other environmental factors. Such disruptions can cause divergence in the output signal during measurement and thus lower the accuracy and precision of biosensor equipment (Bhalla et al. 2016).

This is because the biosensor device's transducers and other electrical components are largely temperature sensitive, which can significantly impact their stability. Temperature can also alter the biological receptor's integrity, as this component degrades with temperature variations (Wilkins and Atanasov 1996).

8.4.4 Detection Limit

It may be defined as the least concentration of target, a quantifiable signal or response. A biosensor's detection limit should be as low as possible (Wilkins and Atanasov 1996).

8.4.5 Reproducibility

Another important feature in biosensing is the capacity of the biosensor equipment to provide the same output signals or outcomes in duplicate runs of the experiment. The biosensor's capacity to achieve this criterion relies on the transducer's ability to act precisely and accurately (Bhalla et al. 2016).

8.4.6 Response Time

This feature describes how long it takes for the biosensor to produce a signal once the biological receptor engages with the target analyte (Njagi and Kagwanja 2011).

8.4.7 Range or Linearity

Linearity of a biosensor can be defined as the precision of the signal received in response to a collection of samples with variable concentrations. This property describes the biosensor's resolution, which is the smallest alteration in the concentration of target analyte required for the biosensor to respond. Because most applications need a biosensor to monitor a target analyte over an extensive range of concentrations, this is a critical feature for a biosensor (Wilkins and Atanasov 1996).

8.5 Classification of Biosensors

Biosensors are classified based on detection system into thermal, electrical, mechanical, magnetic, and optical biosensors. Biosensors are categorized as affinity, catalytic, or non-catalytic biosensors based on the biorecognition principle (Nguyen et al. 2019). In a catalytic biosensor, the contact between the analyte and the bioreceptor leads to the production of a unique biological reaction product. Enzymes, bacteria, tissues, and entire cells are all included in this biosensor. The analyte is irreversibly bonded to the receptor in an affinity (non-catalytic) biosensor, and no new biological reaction product is generated during the interaction. Cell receptors, antibodies, and nucleic acids are used as the targets for detection in this sort of sensor (Iglič and Rappolt 2019).

8.5.1 Enzyme-Based Biosensors

Enzymes are known as biocatalysts that are capable of speeding up biological reactions. The binding capabilities and catalytic reaction for detecting the target analyte are fundamental to the operation of an enzyme-based biosensor (Morrison et al. 2007).

For enzyme-based biosensors, electrochemical transducers are most typically utilized. Glucose and urea biosensors are the most prevalent enzyme-based biosensors. Cordeiro et al., created and characterized enzyme-based biosensors for in vitro real-time glucose monitoring. For enzyme-based biosensors, electrochemical transducers are most typically utilized. Glucose and urea biosensors are the most prevalent enzyme-based biosensors. For enzyme-based biosensors, electrochemical transducers are most typically utilized. Glucose and urea biosensors are the most prevalent enzyme-based biosensors (Cordeiro et al. 2018).

The usage of enzymes as recognition elements in biosensors has expanded as a result of combining enzymes with nanomaterials (Naresh and Lee 2021). Uygun et al. used nanoparticles to create a highly stable potentiometric urea biosensor. Their designed sensor has a reaction time of 30 s and a detection limit of 0.77 _M (Öndeş et al. 2021).

8.5.2 Antibody-Based Biosensors

Antibodies are the elements with biorecognition characteristics having a wide range of applications and have been used for two decades because of strong antigen–antibody interactions. Antibodies have a similar structure to immunoglobulins (Ig), comprising two light chains and two heavy polypeptide chains joined together by disulfide bonds (Dodig 2009; Naresh and Lee 2021).

Immunosensors are biosensors with an implanted antibody as a ligand or rely on antibody–antigen interaction (Skottrup et al. 2008). There are two types of immunosensors: non-labeled and labeled. The complex of antigen and antibody

complex is determined using non-labeled immunosensors by calculating the physical changes that occur as the complex forms. A sensitively detectable label is used in the case of labeled immunosensors. Label measurement is a sensitive way to analyze the antigen–antibody complex (Lim and Ahmed 2019, 2016).

8.5.3 Aptamer-Based Biosensors

Aptamers are short segments of single-stranded nucleic acids that are synthesized and may be folded into 2D and 3D structures and preferentially bind to target molecules. Due to increased surface density and reduced spatial obstruction, the targets have a high binding performance in 2D or 3D structures (Tombelli et al. 2005; Dhiman et al. 2017; Hong et al. 2012).

Because of their nucleic acid nature, aptamers are functionally stable molecules against various storage conditions. Aptamers are stable in a wide range of pH from 2 to 12 and can undergo thermal refolding. Aptamers can be modified chemically per the target molecule's requirement (Naresh and Lee 2021).

8.5.4 Whole Cell-Based Biosensors

Microbes (bacterial, fungus, algae, protozoa, and viruses) are used for developing whole cell-based biosensors because they contain possible biorecognition elements (Riangrungroj et al. 2019. They can also build recognition elements like antibodies necessity for purification and extraction (Gui et al. 2017; Kylilis et al. 2019).

These biosensors are easier to work with and proliferate faster than animal or plant cells. The cell can react with a wide range of analytes, generate the electrochemical signal that a transducer can detect, and communicate the information (Berepiki et al. 2020; Ron and Rishpon 2009).

8.5.5 Nanoparticle-Based Biosensors

Different nanomaterials have been used as bioreceptors as nanotechnology and nanoscience have progressed (Zamora-Galvez et al. 2017). Aside from the bioreceptors listed above, nanomaterials have recently been identified as a new type of bioreceptor. NMs can function as both bioreceptors and transducers. In biosensing technology, NPs have a wider range of uses. Cerium oxide-based nanomaterials, for example, have a catalytic activity beneficial to bioreceptors (Singh et al. 2020). Different inorganic materials, including graphene and CNT-based nanomaterials, noble metal NPs, and QDs, have been effectively exploited as transducers due to their effective transduction properties (Chamorro-Garcia et al. 2016; Liu et al. 2016).

8.5.6 Electrochemical Biosensors

An electrochemical biosensor comprises two main parts: an electrochemical transducer that converts biological data into an electrical signal that is then seen on a reading device (Ronkainen et al. 2010). A signal created on the surface of the electrode is turned into an electrical signal for quantitative measurement following active contact between the analyte and biorecognition element. This type of biosensor can detect infections in the air, water, and seeds in various environments, including greenhouses, postharvest storage conditions, and fields (Grieshaber et al. 2008; Fang and Ramasamy 2015).

8.5.7 Optical Biosensors

Colorimetric biosensors, surface plasmon resonance-based biosensors, and fluorescence-based assays are the most popular optical biosensors for plant pathogen detection. These biosensors quantify the reaction between a target analyte and ligand. Optical biosensors use a light source, an immobilized biorecognition element, an optical transmission medium, and a signal detecting device. Finally, in phase, the amplitude and frequency of a specific light are measured in response to the biorecognition process's physicochemical conversion (change) (Ray et al. 2017).

8.6 Biosensor-Based Diagnosis of Plant Diseases

Plant pathogenic microorganisms produce a variety of infectious diseases that are becoming a significant threat worldwide. Electrochemical approaches for detecting plant infection have raised interest due to their simple instrumentation, high specificity, affectability, speed, and cost, as well as their potential for use in sub-atomic sensing instruments (Yin et al. 2012). Many researchers have attempted to construct various sorts of biosensors using a variety of methodologies, but they have too far.

Umasankar et al. have published nanomaterial-based electrochemical sensors for plant disease detection (Umasankar and Ramasamy 2013). Ariffin et al. described the photolithography-based synthesis of nanowire transducers and their usage in detecting CMV and PRSV viruses (Ariffin et al. 2014; Patel 2021).

In recent decades, considerable advancements have been made in plant disease diagnosis using biosensor-based approaches. Regiart et al. developed a microfluidic electrochemical-based biosensor for detecting *Xanthomonas arboricola* in the walnut plant (Regiart et al. 2017). This method was highly specific, sensitive, and three times faster than ELISA. Malecka et al. used DNA-based biosensor to detect Plum Pox Virus (PPV) (Malecka et al. 2014). An electrochemical biosensor based on an ion channel was used to assess the interaction between ssDNA of PPV on the carbon electrode surface. This electrode had a detection limit of 12.8 pg. Another electrochemical biosensor was developed with the same sensitivity for detecting PPV virus using an anti-PPV polyclonal antibody (Jarocka et al. 2011).

These biosensors are not expensive, need little skill, and are used to identify target pathogens quickly in the field to detect plant infections. The biosensors are also highly selective and sensitive. For example, a nanoparticle electrochemical-based biosensor was more sensitive and accurate than traditional PCR in detecting *Pseudomonas syringae* (Lau et al. 2017).

DNA-based electrochemical sensors are capable of monitoring the environment to detect plant pathogens. Among the various electrochemical biosensors, quartz crystal microbalance-based (QCM), electrochemical impedance spectroscopy (EIS), and voltammetric techniques are used in labs.

Khater et al. developed a labelless biosensor for detecting the citrus tristeza virus and results suggested that this technique can be employed to detect plant diseases in the field. The sensing platform was developed using gold nanoparticles on the carbon electrode to detect the ssDNA layer (Saha et al. 2012; Zhou et al. 2016; Khater et al. 2019). Cebula et al. developed label-free EIS using antibody-modified gold electrodes to detect *Pseudomonas syringae* pv *lachrymans*. Wang and Li developed a microfluidic microarray assembly method for detecting three fungal species: Botrytis squamosa, *Botrytis cinerea,* and Didymella bryoniae, at the same time. They used a glass chip coated with polydimethylsiloxane by using probe line arrays (Hughes 1922). The sample was detected quickly when crossed through the microarray line through microchannels. Lau et al. developed another sensitive approach to detecting the DNA of plant pathogen by using colloidal gold nanoparticles as capture probes (Wang and Li 2007; Lau et al. 2017). This biosensor utilized the recombinase polymerase amplification of the target DNA sequence to detect *Pseudomonas syringae* in infected plant samples. Bhardwaj et al. used gold nanobipyramids optical biosensors for detecting Aflatoxin B1 (Bhardwaj et al. 2021).

Charlermroj et al. developed a novel method to detect four important plant pathogens using microsphere immunoassay: Chili Vein-banding Mottle Virus, *Acidovorax avenae citrulli,* Watermelon Silver Mottle Virus (Tarasov et al. 2015; Charlermroj et al. 2013a, b). Fluorescence-coded magnetic microspheres and antibodies are used to detect the target pathogen, and further evaluations were made using antibodies labeled with R-phycoerythrin (Ashiba et al. 2017; Charlermroj et al. 2013a, b). Adegoke et al. developed quantum dot based nanobiosensors to detect ZIKV RNA (Adegoke et al. 2017).

8.7 Biological and Technical Limitations in Detection Using Biosensors

Due to recent breakthroughs in micro-and nanotechnologies, biosensor-based detection methods are extremely specific, sensitive, and offer speedy findings. However, there are other factors to consider and potential obstacles in adopting and implementing these techniques in agriculture. Biosensors will become an excellent tool for plant disease management, but these should be applied with information on the crop, pathogen, and disease epidemiology. This information includes specific characteristics, cultural practices, environmental conditions, and various

management strategies. This could lead to the development a prediction system that could be employed to enhance crop productivity and quality (Khasanov et al. 2009; Dominguez et al. 2015).

Furthermore, the detection accuracy using biosensors depends on various sampling techniques such as sampling design, duration of sampling, and various methods which expose the analyte to a biosensor probe (Ray et al. 2017).

The biological quantities of target pathogens in plant materials evaluated may significantly impact detection limits. Sometimes they are interrupted by the presence of non-target molecules that interfere with probe binding (Wei et al. 2010).

When nucleic acids are used as a target for hybridization of complementary capture probe, the process impacts binding specificity and accuracy in the diagnosis, which could lead to false-positive results due to nonspecific capture on the sensing surface. The complexity of the sample matrix, which incorporates ions and cells, could cause amplifier optimization to fail (Wang et al. 2017; Vidic et al. 2019). Separate the target analyte from the sample is a critical step in improving detection (Sin et al. 2014).

Despite the various advantages of using optical and electrochemical biosensing approaches over the conventional methods discussed in this study, more research is needed to apply this technology for quantifying plant pathogens in the field. Validation of a portable sensor necessitates using specialist hardware, which can be costly and difficult for non-experts like farmers. It is still unknown how much inoculum is required to cause disease in host plants. This threshold must be established to transform the pathogen level measured by the biosensor into an estimate of disease risk.

8.8 Future Work

Even though plant biosensors-based research has become increasingly important, there is now less study available. Furthermore, the expected presentation of biosensors for its application on non-plant entities does not exclude plant biosensors from moving in the presence of current processes such as "Transcriptomic Biosensors," "Hereditarily Encoded Biosensors," and "ChmericBiosensing technologies." Incorporating unique disease detection sensors, such as optical fiber biosensors and electrochemical biosensors, would provide a more beneficial technique to detect plant infections.

Given the importance of plant health monitoring, the current state of biosensors, and the limitations of currently available techniques and novel biomarkers, scientists are expected to refocus their efforts on plant health and growth monitoring with an appropriate kick start.

8.9 Conclusion

One of the most commonly encountered problems worldwide is the loss of a significant portion of crop yield due to diseases and pests. The presence of diseases in the plants reduces the output after supplying adequate resources to the fields. This

set of prompts focuses on practical approaches for identifying the cause of plant diseases. Early identification of the type of plant disease present is required to provide powerful strategies for diagnosing and avoiding destruction. Traditional methods for detecting phytopathogens, such as microscopic analysis of diseased tissue or culture, take time and require a knowledgeable person, but emerging techniques, such as biosensors, are simple and quick. Biosensors for identifying plant pathogenic bacteria have sparked increased attention, with research focusing on creating compact portable devices that would allow for quick, precise, and localized recognition. Agriculture scientists should investigate biosensor technologies in conjunction with synthetic biology.

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Immunotechnology for Plant Disease Detection

9

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Abstract

There has been a persistent concern that massive crop production loss occurs yearly due to plant pathogens worldwide. Developing and using advanced technologies for timely plant disease detection are vital to reduce crop damage by pathogen infection during crop development, harvesting, and post-harvest processing of the produce, boosting crop productivity and ensuring agriculture sustainability. Among various techniques available to detect plant disease, immunotechnology is broadly recognized as the most effective and powerful disease diagnostic tool. Immunotechnology is generally popular for its quick, highly sensitive, particular features that make it a reliable tool for pathogen detection. However, proper application of such techniques is very challenging as it requires a substantial cost for equipment as well as one has to thoroughly understand the technology to deduce and troubleshoot irregular errors or lab results. In this chapter, we have demonstrated in detail the application of

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immunotechnology for detecting plant pathogens and highlighted the advantages and limitations of each technique based on immunotechnology.

Keywords

Disease diagnostic tool · Immunotechnology · Detection of pathogens · Inoculum quantification · Advantages and limitations

9.1 Introduction

Management of plant diseases is a top issue in economies that rely heavily on agriculture due to the significant agricultural output lost annually due to various diseases, a particularly acute problem in developing nations (Agrios 2005; Strange 2012). Approximately, US $1500 billion is spent annually on agriculture (Agrios 2005). During the unavailability of resistant plant varieties, the most effective alternative is to detect and quantify the pathogen as early as possible and prevent the infection's development. So, the use of quick, accurate, and targeted diagnostic techniques is crucial for the success of several integrated pest control schemes. Developing a more practical approach for plant disease detection and linking them to decision-making authorities to successfully detect plant diseases and protect crop production are critical. To detect plant diseases, several methods have been designed (Punja et al. 2007).

Immunotechnology is a successful way of quick and accurate pathogen detection, but each technique has some limitations and drawbacks in addition to advantages. Immunotechnology includes various techniques such as serological assays (i.e., antibody-based techniques), nucleic acid-based techniques, and point-of-care DNA extracting techniques. Early traditional approaches relied on symptom observation, including field examinations to diagnose disease symptoms and analyze pathogens in the lab by cultivating specific culture media following biochemical, physiological, and pathogenicity assays (Horsfall and Cowling 1977). While traditional procedures are effective, they take a long time and need experienced or professional plant pathologists to detect the pathogen causing the particular disease. For diagnosing plant pathogens, it was important to find detection techniques with improved specificity, sensitivity, and speed (Ul Haq and Ijaz 2020). Therefore, immunotechnology has gained success in rapidly detecting plant diseases and offering pathogen management at an early stage.

Due to their efficiency, sensitivity, and low cost, serological diagnostic techniques for the detection of plant diseases have gained popularity. Since the 1980s, the use of antibodies in plant diagnostics has increased, and several evaluations of this technique have been reviewed (Alvarez 2004; Narayanasamy 2011). The poor specificity of polyclonal antibodies for phytopathogens formed by animal vaccination may cause them to interact with adjacent pathogen species (Macario and Conway de Macario 1985). As monoclonal antibodies only target a single antigen in a pathogenic protein, sensitivity has increased due to their

development (Thornton 2009). As a result, several serological diagnostic techniques have been designed and are often used to detect plant diseases, including enzyme-linked immunosorbent assays (ELISA), lateral flow devices (LFD), immunofluorescent tests, and immunoblots (Comstock 1992; Novakova et al. 2006; Kokoskova and Janse 2009; Tomlinson et al. 2010). The production of monoclonal antibodies is costly, though, and it has been suggested that related species may have similar epitopes that induce monoclonal antibodies to respond favorably (Teitelbaum et al. 1991; Gorris et al. 1994).

Researchers were able to research and design nucleic acid-based methods to detect plant disease due to the development of the polymerase chain reaction (PCR) in the 1980s. Several PCR-based screening techniques are now for detecting plant pathogens (Ward et al. 2004a, b; Vincelli and Tisserat 2008). For instance, immunocapture-PCR (IC-PCR), a specific device, which combines standard PCR amplification with infectious viral particles that antibodies have trapped, has been used to identify viruses. This method effectively identified the bacterial blight disease (caused by Xanthomonas axonopodis pv. dieffenbachiae) in Anthurium propagation material and enhanced sensitivity by 250-fold than direct amplification by conventional PCR (Khoodoo et al. 2005). To increase sensitivity and specificity, it has also been documented to amplify pathogen-specific sequences and couple PCR with other methods (Merighi et al. 2000; Brasileiro et al. 2004; Carrasco-Ballesteros et al. 2007).

Early in the 1990s, PCR-ELISA, a fusion of traditional ELISA and PCR, was established (Nutman et al. 1994). An ELISA plate with an immobilized probe and a labeled PCR product are hybridized together in this test. Subsequent inclusion of an enzyme conjugate and a substrate allows photometric analysis of the acquired PCR product. With this test, one amplicon community, among many others produced in a multiplex reaction, may be found (Laitinen et al. 2002). With more specificity than traditional PCR, this method has been used to detect bacteria effectively, fungi, and viruses (Shindo et al. 1994; Daly et al. 2002; Bailey et al. 2002; Somai et al. 2002). The DNA amplified by PCR was discovered to crossbreed with the ELISA probe from other microbial species, which is why the test produced false-positive findings when identifying Mycobacterium tuberculosis and Neisseria meningitides (Kent et al. 1995; Gillespie et al. 1997; Borrow et al. 1998).

Real-time PCR that enabled the prevalence of pathogens to be detected and their quantification in plant tissue marked a significant advancement in DNA-based diagnostic procedures. This allowed assessing the disease severity (Heid et al. 1996). The requirement of this method for pricey tools and reagents prevents it from being used as a quick, inexpensive diagnostic approach to immunotechnology. Additionally, due to the high sensitivity of this method, there is a probability that it will detect even minute quantities of contamination in biological materials or reagents, leading to the assessment of false positives and necessitating the use of standardization procedures or pre-read runs to ensure the reliability of the findings (Wong and Medrano 2005; Nowrouzian et al. 2009).

Point-of-care diagnostic tests are an important diagnostic approach based on immunotechnology that may be carried out quickly and affordably in the open

field without using expensive equipment (Yager et al. 2006). Although PCR-based techniques offer several benefits over competing technologies, they are significantly less suitable for POC applications because they need the energy to execute the temperature variation necessary for DNA amplification (Barany 1991). Isothermal DNA amplification techniques work well to overcome this restriction (Gill and Ghaemi 2008; Craw and Balachandran 2012). For example, POC diagnosis of plant disease has been accomplished using isothermal amplification in conjunction with portable fluorometers and lateral flow strips for DNA extraction (Vincent et al. 2004; Piepenburg et al. 2006; Chow et al. 2008; Lutz et al. 2010; Mahalanabis et al. 2010; Rohrman and Richards-Kortum 2012). However, the cost and potential suitability of portable fluorometers for usage in harsh weather-prone environments prevent their wide-scale use. Finding a POC diagnostic assessment technique that integrates the complete procedure from sample processing to result reporting is still difficult. Given that field might be far from bioanalytical labs and that adequate transportation can provide logistical challenges, the agricultural sectors could benefit immensely from the availability of practical and affordable POC methods. This chapter emphasizes various techniques for plant disease detection based on immunotechnology.

9.2 Immunotechnology to Detect Plant Diseases

9.2.1 Immunotechnology Based on Serological Methods

Serological methods were designed to identify viruses because they cannot be spontaneously grown. Monoclonal and polyclonal antisera and methods like western blots, enzyme-linked immunosorbent assay (ELISA), dot-blot immunobinding assay, immunostrip test, and serologically specific electron microscopy (SSEM) can now be used to detect more than a thousand pathogens, bacteria, and fungi (Alarcon et al. 1990; Caruso et al. 2002; Van Vuurde et al. 1987; Hampton et al. 1990). Due to its high-throughput capacity, ELISA, which was first utilized in the 1970s, is perhaps the most popular immunodiagnostic method. The organism, freshness of the tested sample, and titer affect how sensitive an ELISA test is; for example, bacteria can be detected at 100 cfu/mL (Schaad et al. 2001, 2003). Antibodies cannot be frequently defrosted and must be stored at temperatures lower than −20 °C.

Many bacteria and viruses have polyclonal antisera that have been developed in research laboratories or for commercial purposes and used in various techniques (Nolasco et al. 2002). However, due to their recurrent cross-reactivity, monoclonal antisera that are more potent have been formed through the hybridoma technique with cell lines specific to single epitopes (Holzloehner et al. 2013). ELISA techniques using monoclonal and polyclonal antibodies are already developed for several taxa of plant pathogenic bacteria. Reliable diagnostic kits are being marketed commercially. Monoclonal antibodies are more costly and often detect just one antigen. Numerous epitomes on a single antigen are detected by polyclonal

antibodies, which are also inexpensive than monoclonal antibodies (Robison 1995). They do, however, have a limited shelf life and batch variations.

9.2.2 Immunotechnology Based on Nucleic Acids

Fluorescence in situ hybridization (FISH) and the numerous PCR versions real-time PCR (RT-PCR), nested PCR (nPCR), multiplex PCR (M-PCR), cooperative PCR (Co-PCR), and DNA fingerprinting) all are immunotechnology-based nucleic acid techniques for the detection of plant diseases. Nucleic acid sequence-based amplification (NASBA), AmpliDet RNA, and reverse-transcriptase PCR are RNA-based alternatives. These techniques can get beyond ambiguous pathogen taxonomy or diagnoses, allowing for quick and precise pathogen detection and quantification (López et al. 2009). The preparation of samples for molecular characterization is crucial and demands effective and repeatable techniques. Numerous documented DNA and RNA isolation techniques were designed to prevent the occurrence of inhibitory substances that make pathogen detection more difficult (Louws et al. 1999). Humic substances, phenolic compounds, or polysaccharides from plants or other substrates are the main components that impede DNA polymerase (Minsavage et al. 1994; Mumford et al. 2006).

Numerous methodologies have been generated for PCR-based techniques employing various genomes, such as dsDNA, ssDNA, or ssRNA. It is a common practice to use commercial kits especially developed for extracting nucleic acids from different plant materials. The Ultra Clean Plant DNA and RNA extraction kits from MoBio, RNeasy and DNeasy Plant System from Qiagen, and the Extract-N-Amp Plant PCR kit and Easy-DNAExtraction kit from Sigma are among the standout products. Sonication or electrical devices may be integrated into Lab-On-A-Chip devices to treat and purify the genome (Taylor et al. 2001; Gascoyne et al. 2004). The most recent technology uses small tools for DNA isolation from microchips by techniques like laser irradiation and capillary electrophoresis (Lin et al. 2007). Although intriguing, not all plant materials respond well to these techniques. Before being used for regular detection, they must be tested against every possible plant, pathogen, or substrate combination (López et al. 2009; Ul Haq and Ijaz 2020).

Precisely designing probes and oligonucleotides is the foundation of all molecular diagnostic techniques for plant disease detection. The National Center for Biotechnology Information (NCBI) provides GenBank® (a Nucleotide Sequence Search tool) to locate the target sequences. The Basic Local Alignment Search Tool (BLAST), with the BLASTn tool built to explore nucleotides, can be used to find similar sections for every target. Primer designs for RNA or DNA targets are simple to create when specific nucleotide sequences are chosen (Ul Haq et al. 2021; Babar et al. 2021; Nasir et al. 2021). Various molecular techniques are available to detect plant pathogens in open areas, greenhouses, and orchards. One of the most popular vegetable species worldwide is the tomato (*Solanum lycopersicum* L.), grown on over 4.6 million hectares globally (FAO). Due to the ongoing and unabated need

from the processing sector and consumers, this crop is being grown in monoculture. This substantially encourages the colonization and pathogenicity development of many diseases. The global horticulture issue is getting severe due to the increased prevalence of several endemic plant diseases and the advent of new ones brought on by various pathogens, including viruses, bacteria, fungi, and phytoplasmas. The tomato yellow leaf curl disease in Sicily (TYLCD) has been reported to be caused by another viral agent known as Begomovirus but appears to generate the same symptoms in plants and is one of the most significant cases. The loss of crop production might range from 20 to 100% based on the specific Begomovirus involved. It implies that immunotechnology is the only approach for differentiating the several Begomoviruses (Davino et al. 2006).

Compared to immunoassays, PCR has several benefits, including the potential to identify a single target in complicated mixtures, the quick and accurate detection of many targets, and the possibility to detect obligate plant pathogens that cannot be cultured as viruses, certain bacteria, and phytoplasma (Ijaz et al. 2022). For amplification and detection of DNA in PCR-based diagnostic tests, primers are produced to bind with particular DNA segments from the target species. Plant diseases are frequently detected and identified by amplifying specific sequences of nucleic acids (Mumford et al. 2006). The pathogen is present in the analyzed sample, and the detection of the amplification material confirms its presence. SYBR GREEN UV light detection has superseded the original approach of seeing the amplified product by agarose gel electrophoresis involving ethidium bromide (EtBr) staining. The application of dye-quenched probes has increased PCR accuracy and pathogen sensitivity (Morris et al. 1996; Thelwell et al. 2000). Although more advanced immunotechnologies can achieve the desired result within minutes, PCR can typically be completed in 2–3 h (Tomlinson et al. 2007). The most practical method for detecting plant viruses is RT-PCR, which has a sensitivity comparable to ELISA or hybridization approaches.

With nPCR, which is carried out in two phases and uses either 1 or 2 internal primers, specificity and sensitivity can be improved (Simmonds et al. 1990; Olmos et al. 1997; Pradhanang et al. 2000). Primer interaction between the two amplification may result from this strategy. Additionally, contamination rates may rise with two sessions of amplification in several tubes when using the procedure frequently and extensively.

Co-PCR was created to identify plant pathogenic bacteria and viruses (Caruso et al. 2003). Co-PCR, performed in a single reaction, lowers the possibility of contamination and provides sensitivity comparable to RT-PCR and nPCR. The specificity for virus diagnosis when colorimetric detection is at least 1000 times greater than that achieved with RT-PCR and is comparable with those of nested RT-PCR. The nucleotide sequencing can be described by combining it with dot-blot hybridization. But the limited reagents can make the reaction more susceptible to inhibitors, demanding better RNA quality (Olmos et al. 2005).

M-PCR is a PCR variant amplifying two or more target sequences instantaneously in the same practice (López et al. 2009). As many diseases typically attack a single plant, M-PCR is helpful (Davino et al. 2012; Panno et al. 2012, 2014).

Experimental evaluation of the selected primers reliability is required. Combining the benefits of M-PCR and nPCR, the multiplex nested RT-PCR speeds up the process and lowered costs in a single tube while enabling simultaneous detection of the target pathogen. Similar to M-PCR, it involves an in silico evaluation and experimental confirmation of primer reliability. As opposed to traditional PCR, RT-PCR enables pathogen detection and real-time reaction analysis. Numerous specialized techniques have been devised, and this high-throughput method delivers excellent speed, sensitivity, specificity, and reliability. Using modified primers, distinct primer tags, and combined probes, RT-PCR eliminates the potential of cross-contamination. However, RT-PCR needs to be modified to account for elements like reaction components, circumstances, and primer design. Compared to conventional PCR, it performs well with tiny amplicons (50–200 bp) and at lower dNTP, primer, and magnesium concentrations. Several chemistries, including TaqMan, SYBRGreen, Molecular Beacons, and Scorpion, are accessible commercially. For each analysis, the type and design of the primer must be tuned. Since TaqMan can distinguish between nucleotide sequences that vary just by one bp, it is the most popular real-time PCR system.

DNA fingerprinting is a molecular nucleic acid-based technology used to identify people based on distinctive patterns (polymorphisms) in their DNA by using samples of blood, hair, semen, or other biological materials (Ijaz 2011). The method was first introduced in 1984, focusing on DNA sequences known as mini-satellites that featured sequences with an unknown purpose. Apart from identical twins, these patterns are particular to each person. This method was modified for the detection of plant diseases. Targeting regions of DNA with defined variability in single nucleotides (short tandem repeats, STRs; single-nucleotide polymorphisms, SNPs) or other repeating polymorphic zones is done by various DNA fingerprinting techniques using either PCR, restriction fragment length polymorphism (RFLP), or both. The quantity and length of recurring patterns evaluated determine the likelihood of successfully identifying an individual.

Most fingerprinting techniques use PCR to detect fragments. These include amplified 16S ribosomal DNA restriction analysis (ARDRA), repetitive-sequence PCR (rep-PCR), random amplified polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE), and amplified fragment length polymorphism (AFLP) (Grothues and Rudolph 1991; Scholz et al. 1994; Clerc et al. 1998; Little et al. 1998; Scortichini et al. 2001; Manceau and Brin 2003). The application will determine the approach, including diagnosis, DNA marker tracing, the organism being studied, and the plant–pathogen interaction. A fingerprinting method should typically not require any prior investment in DNA probe characterization, sequence analysis, or primer design. One of the most accurate genetic approaches for differentiating between plant pathogenic bacteria is AFLP analysis (Vos et al. 1995). AFLP uses PCR amplification to determine nucleotide restriction fragments for DNA of any source or intricacy. A small number of universal primers are used to create fingerprints with not any prior sequence information. Choosing particular primer sets makes it possible to "tune" the number of fragments identified in a single reaction. Since the AFLP approach combines the RFLP dependability with the PCR

power, it is durable and reliable due to the strict reaction conditions utilized for primer annealing. Fluorescent dye-labeled primers and automated DNA sequencers for data collection have recently been added to the methodology, which reduces the complexity of the original AFLP technology. To effectively detect and classify bacterial species, this modified technique (fAFLP) has been applied (Manceau and Brin 2003; Cirvilleri et al. 2007a, b).

RNA sequences are frequently amplified using NASBA. For regular amplification of genome in a single composition at a single temperature, this technique was introduced in the early 1990s (Compton 1991). Just a water bath is required by NASBA and does not need a thermal cycler. This method has been used to detect bacteria and viruses (Rodriguez-Làzaro et al. 2006; Olmos et al. 2005; Klerks et al. 2001; Scuderi et al. 2010). DNase treatments are also not required because NASBA amplifies RNA. This method may be used to precisely identify live cells by selectively amplifying mRNA sequences against a backdrop of genomic DNA. The reagents and time needed to produce the amplicons can be reduced using a hybridization device (such as Hybrio Ltd. or Hybrimax) (Olmos et al. 2005).

Nucleic acid detection underwent a renaissance in the early 1990s due to DNA arrays. Target sequences were quickly and accurately determined. Support miniaturization and greater spot density were made possible in the previous 2 years by better visual scanning. The size of resultant chips or "microarrays" is just a few cm^2 and can contain millions of probes representing a target species' whole transcriptome or genome. PDD has made considerable use of oligo DNA microarrays, which imprint the spots utilizing a variety of oligo lengths and methods (Singh-Gasson et al. 2000). The oligos size ranges between 25 and 70 bp. Although small oligos are more effective at detecting non-specific hybridizations, large oligos often have greater sensitivity (Bates et al. 2005).

For phytopathogens, detecting microarrays have been created through several worldwide collaborations. The procedure enables the direct performance of many molecular diagnostic procedures, including isolation of genome, PCR reactions, and pathogen detection, with the possibility for automation (Liu et al. 2007; van Doorn et al. 2007; Ijaz et al. 2022). Additionally, microarrays are capable of multiplex pathogen detection.

The number of target species, the study's length, and the project's budget are only a few variables that influence the selection of a molecular approach (López et al. 2009). Although these techniques are quite effective and precise, they have some drawbacks. For example, unequal distribution of pathogens throughout plants, especially woody perennials, might make molecular diagnostics unreliable, especially at stages before symptom development. Pathogen titers are frequently under the sensitivity level of these assays, typically 10–100 pg, in substances including water, insect vectors, seeds, and soil. When the target sequence of nucleic acid is damaged, or the reagents are of poor quality, false-negative results can also happen (Louws et al. 1999). Sample contamination and the small size of the sample both have the potential to produce false-positive results. Because of polymerases and transcriptase inhibitors, the specificity of PCR-based procedures is frequently lower than anticipated. Deceased plant pathogens can provide false-positive findings in

PCR, which is especially important when examining quarantine diseases. Deceased pathogens can also amplify non-specific compounds and artifacts due to incorrect priming or dimerization. Furthermore, apparatus and reagent costs must be considered while choosing an immunological technique.

9.2.2.1 Advantages

There is no denying the advantages of speed, high specificity, sensitivity, and the capacity to measure pathogen biomass. Here are a few instances of how nucleic acid (NA)-based tests have been used.

1. Quickly identifying and detecting infections for which conventional methods are insufficient or unavailable, such as:
   (a) Obligatory parasites, particularly in samples of a complex environment—for instance, vascular arbuscular mycorrhizae (Henson and French 1993; Chen and Zheng 2007; Liu et al. 2015);
   (b) Species that need substantial expertise to detect through microscopy, e.g., *Meloidogyne* spp. (Stanton et al. 1997; Tesařová et al. 2003);
   (c) Species that cannot sporulate or develop poorly under in vitro conditions (Tisserat et al. 1994);
   (d) Pathogenic microbes that resemble associated nonpathogenic microbial species morphologically (Peres et al. 2007);
   (e) Immature life phases of pathogens, for example, eggs or juveniles of nematodes (Powers 2006; Adam et al. 2007).
2. The detection and characterization of pathogens may be of regulatory significance or have a significant economic impact. Sudden oak death is caused by the pathogen *Phytophthora ramorum* (Tooley et al. 2006; Bunyasi et al. 2022). Karnal bunt of wheat due to *Tilletia indica* infection and citrus canker caused by *Xanthomonas citri* strains are three significant instances of plant diseases where molecular characterization is a vital technique (Frederick et al. 2000).
3. Measuring biomass of the pathogen in samples of ambient material or host tissue (Mumford et al. 2006).
4. Determining the geographical origin of disease strains or the genetic links (Fatmi et al. 2005).
5. Keeping track of latent infections presence and comparative significance. NA-based approaches can be used with histological assessments to distinguish between restricted, latent infection in symptomless plant tissues and active colonization of the host plant by the virulent pathogen (Flowers et al. 2006).
6. Detecting is still alive but no longer culturable bacteria (Grey and Steck 2001).
7. Detection of primary inoculum of the airborne pathogen in the early period (Bunyasi et al. 2022), without requiring persons expertized in morphological detection of pathogenic fungi.
8. Evaluation of pathogen for host plant indexing and certification programs (Weller et al. 2000).
9. Identifying and measuring seed-borne pathogens (Pecchia et al. 2019).

10. Screening biotypes of aggressive pathogens belong to different races (Mavrodieva et al. 2004; Hariharan and Prasannath 2021).
11. Fungicide resistance analysis includes measuring the number of resistance alleles of fungicide in a pathogen population (McCartney et al. 2003; Zhonghua and Michailides 2005).
12. Evaluating a cultivated crop probable risk of storage degradation (Atallah and Stevenson 2006).
13. Screening biomass of pathogen and plant colonization are involved in breeding programs to differentiate between hosts plants that are tolerant and those that are resistant (20).

9.2.2.2 Limitations

There are several review publications on NA-based methods for microbe identification, and the benefits of these methods are well-established and well-explained. Fewer reviews go into depth on limitations, but people still need to be aware of them to utilize NA-based detection techniques correctly.

9.2.2.2.1 False Positives and Negatives are Brought on by Improper Specificity

Operators of NA-based techniques must understand that primers and probes may not always show complete specificity for the particular pathogen and just that pathogen—in each experiment. An isolate's primary collection is utilized to design a primer set that may not have included every naturally present variant or clade, which might result in false positives PCR (Cuppels et al. 2006; Tredway 2006; Rubio et al. 2020). Amplification of DNA may not occur if the primer-binding site is altered, even by a single-nucleotide mismatch (Singleton 2000; Stadhouders et al. 2010). A primer set intended to be species-specific or strain-specific may, on the other hand, cross-react with other strains or species, leading to false-positive results (Weller et al. 2000; Tredway 2006; Lauri and Mariani 2009). NA-based assays are not the only ones with imperfect specificity, but it is crucial to be aware of this risk when performing these assays.

Using molecular primers or markers created for a minimum of two distinct genomic regions strengthens NA-based pathogen identification techniques according to the principle that two sources of evidence are more reliable than one. This is crucial for pathogens with significant potential for injury, specifically when there are few or no non-NA-based sources of evidence available. Results from established NA approaches that fall short of this requirement should be taken with some care. In direct relation to the magnitude of the database used to design and validate a primer set, diagnosticians' confidence in its specificity might rise. The sequences from several isolates reflecting a large geographic range for the target pathogen should preferably be used to design a primer set. However, it is practically unattainable to have a complete illustration of a phytopathogen population before phylogenetic analyses evaluate the connections between strains, as they helped ensure that all relevant clades are included in the sequence alignments utilized to choose primers. The best way to verify primer sets is to evaluate them against

1. Concerned pathogen isolates,
2. A broad range of associated species microbes, and.
3. Distinct species of unrelated microbes and frequently found close to the host tissue that will likely be examined.

Positive responses should always be seen in the first category and not in the second or third group (Vincelli and Tisserat 2008).

9.2.2.2.2 Collection of Samples

The collection of a suitable sample is considered "the most crucial phase in the assessment procedure" in the medical pathology laboratory, and this is also applicable for the detection of plant diseases. Because an NA-based analysis normally only uses one to two microliter of a sample extract, extreme caution must be taken to ensure that the extract is obtained from well-chosen, relevant tissue samples having the most probability of being invaded by the target pathogen (Alvarez 2004; Vincelli and Tisserat 2008). A single worldwide assay for plant pathogens won't be feasible whenever various tissues are needed to screen for various pathogens, even though multiplexing capacities are considerably expanding, mainly through microarrays. At a minimum, one might need a root assay, a lower stem assay, and a foliar assay to test for a wide variety of known pathogens. However, since different pathogens often colonize different tissues and portions of plant organs (Agrios 2004), one might need to separately process and test several samples even if they are collected from the same organ.

Recent survey procedures show potential sample issues for NA-based diagnostic techniques for sudden oak death caused by oomycete *P. ramorum*. This pathogen causes catastrophic oozing cankers on Fagaceae family members, but it can also infect a broad range of plant species by causing foliar spotting, foliar blighting, and/or twig dieback (Davidson et al. 2003). Additionally, *P. ramorum* can invade the potting soil of asymptomatic or uninfected plants (Bulluck et al. 2006; Linderman and Davis 2006). Accordingly, a researcher may gather and analyze several symptomatic samples of infected plant materials and soil to see if P. ramorum exists in a nursery to assure the highest probability of pathogen detection.

9.2.2.2.3 Risks of Contamination

PCR has the exponential nature of target DNA amplification, making the method susceptible to contamination. This emphasizes the requirement for superior lab practice record and performance and incorporating appropriate controls in all experiments. A negative control, which contains water rather than the template, and positive control, which includes DNA from a strain known to respond favorably, should be included in every run. (Espy et al. 2006; Titov et al. 2015; Hu 2016).

9.2.2.2.4 Inhibition of PCR

Inhibitors of PCR include chemical and inorganic substances found in host plant tissues, microbial cell components, and other substances (Schrader et al. 2012; Acharya et al. 2017). To ensure that a negative result is not associated with the

inhibitors present in the reaction tube of PCR, it is crucial to involve appropriate controls with every sample (Wilson 1997; Pearce 1998). It can be achieved using either of 2 basic strategies:

1. An internal control, having a multiplex reaction mixture involving a primer set for the target pathogen and a primer set for the detection of the host genome, e.g., host ITS gene or a cytochrome oxidase gene (Ward et al. 2004a, b; Roux et al. 2019).
2. A parallel control, having the same reaction tube comprising a sample aliquot "spiked" with DNA of an identified positive microbial strain (Singleton 2000). PCR inhibition occurs when the internal or parallel control fails to amplify effectively.

   There are many methods to avoid PCR inhibition, including reducing the amount of plant host tissue that is used to extract pathogen DNA, 10- to 1000-fold dilution of the sample, centrifugation for pathogen concentration, the inclusion of inhibitor-binding substances, cells immunocapture or adsorption of agar to split pathogen cells from inhibiting chemicals, pathogen accumulation on artificial growth media before PCR, and other methods (Ozakman and Schaad 2003; Mavrodieva et al. 2004; Lauri and Mariani 2009; Narayanasamy 2010; Acharya et al. 2017). Although PCR inhibition is a risk that the technician should be conscious of at all times, it is easily handled and provided reasonable controls that allow its diagnosis are involved in every experiment.

9.2.2.2.5 Viability of PCR Positive

Amplification of all DNA template sources occurs in the reaction chamber in PCR-based assays, regardless of whether they are viable. However, technological ways to avoid this issue include sample processing with ethidium monoazide, which covalently binds with DNA and limits PCR amplification by penetrating dead cells (but not living cells). Pathogen selective concentration on medium before PCR processing (Vincelli and Tisserat 2008) and application of reverse-transcriptase PCR (RT-PCR) for the amplification of mRNA, which is linked with living cells, are other methods to improve the diagnosis of viable cells (Singleton 2000). Since assays based on immunotechnology can produce comparable results, the issue of false positives caused by dead microbial cells is not specific to PCR (Janse 1988).

9.2.2.2.6 Other

There are additional potential sources of such failures in addition to the likelihood of false negatives brought on by DNA polymorphisms among conspecific isolates, which was previously described. For instance, if the target pathogen exists as resilient dormant structures, e.g., oospores, from which DNA extraction is difficult, a false negative may occur (Martin et al. 2000; López-Alvarado et al. 2014). The above-discussed measures to improve sensitivity may be helpful if such potential occurs. False-negative results may also happen as diseased tissues deteriorate in vivo due to declining pathogen densities and secondary organism invasion (Cuppels et al. 2006). Last but not least, hybridization between similar pathogen species might

make NA-based diagnoses more difficult (Cooke et al. 2000; Martin and Tooley 2004; Vincelli and Tisserat 2008).

9.3 Conclusion

The number of methods available for tackling disease issues that are now of concern in plant pathology programs is growing because of immunotechnology for detecting the pathogen. The use of immunotechnology nowadays also improves the foundation of the laboratories and the technical capability to react to new problems as they arise. Considering the limits of each approach based on immunotechnology is essential for practical application. The critical application of such tools also requires respect for the importance and utility of conventional laboratory procedures and the need to gather several lines of evidence in persistent situations.

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Molecular Phytopathometry

10

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Abstract

Phytopathogens are naturally occurring organisms that cause severe damage to plant health, resulting in reduced crop quality, productivity, and yield. These pathogens cause a significant threat to the agricultural sector worldwide, leading to severe economic losses. Early detection and identification of these phytopathogens are critical in facilitating effective disease management. Nucleic acid-based detection techniques have proven to be effective in pathogen identification and disease quantification. Recent advancements in several variants of polymerase chain reaction (PCR) assays, DNA or RNA probe-based methods, post-amplification methods, isothermal amplification-based methods, RNA interference, next-generation sequencing methods, and DNA barcoding have a high potential for the identification, characterization, and quantification of phytopathogens. These molecular tools are helpful for the diagnosis of both asymptomatic and symptomatic diseases caused by phytopathogens in their culturable and unculturable states. The detection is based on genotypic characters rather than phenotypic characters as in conventional methods. Hence, these tools are considered more reliable, easier, rapid, highly sensitive, and specific than the conventional methods.

Keywords

Phytopathogens · PCR assays · DNA barcoding · RNA interference · Next-generation sequencing

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10.1 Introduction

Plants are under constant and growing threat from phytopathogens. Phytopathogens are naturally occurring organisms that cause severe diseases in plants. These pathogens cause devastating damage to plants, negatively impacting agricultural food production worldwide. Phytopathogens include fungi, bacteria, viruses, oomycetes, nematodes, parasites, and phytoplasmas (López et al. 2009). It has been estimated that annual agricultural crop losses due to phytopathogens are approximately 2020 billion dollars. FAO (Food and Agriculture Organization) declared the "International Year of Plant Health (IYPH)" for 2020, which aims to improve plant health for the sustainable food industry (FAO 2020). Currently, phytopathogenic fungi cause 83% of loss in the global agricultural sector, followed by viruses, phytoplasmas, and bacteria that account for 9% and 7%, respectively (Khakimov et al. 2022). On average, pathogen-induced yield losses are estimated to be around 12% in soybean, maize, rice, and barley, 24% in potatoes and groundnuts, and 50% and 80% in wheat and cotton, respectively. Post-cultivation losses due to these pathogens are predicted to be 30–40% (Pimentel et al. 2005; Roberts 2006).

Fungal pathogens are predominant infectious organisms that cause severe stress in crops. About 8000 fungal species are linked to plant diseases (Ijaz et al. 2021a, b, c). They can damage plants alone or by interacting with other phytopathogens at any stage, from seedling to mature. Most commonly known fungal diseases are blight (caused by *Phytophthora infestans*), wilt (caused by *Fusarium* and *Verticillium species*), anthracnose (caused by *Colletotrichum spp.),* powdery mildew (caused by *Podosphaera xanthii*), scab (caused by *Cladosporium cucumerinum*), dieback (caused by *Phytophthora spp*), rust (caused by *Puccinia triticina*), leaf spot (caused by *Alternaria, Cercospora*, and *Colletotrichum spp.),* root rot and damping-off (caused by *Pythium*, *Phytophthora*, *Fusarium*, and *Rhizoctonia spp.)* (Iqbal et al. 2018; Jain et al. 2019; Hussain and Usman 2019; Ul Haq and Ijaz 2020; Anjum et al. 2020; Naeem et al. 2020; Ul Haq et al. 2019). It is the foremost requirement to identify diseases caused by these pathogens and evaluate disease severity to overcome food insecurity and economic crisis (McCartney et al. 2003; Ul Haq and Ijaz 2020; Ijaz et al. 2020).

The term "Phytopathometry" or "Plant Pathometry" was first devised by Large (1953, 1966) from Pathos, means "disease" and metron, means "measure." Nutter et al. (1991) Defined Phytopathometry as "disease assessment, " which is an extension of phytopathology that involves assessing and measuring plant diseases. This branch broadly includes exposure, identification, and quantification of diseases caused by various phytopathogens. There are five requirements of disease quantification necessary for yield loss analyses stated by Large (1966) and restated by Gregory (1982). These requirements are (1) detailed information on a healthy crop morphology and its development; (2) extensive study of the plant diseases present in the field; (3) preparing field assessment key for disease severity; (3) performing field trials to assess disease progress using filed key; and (5) assessment of disease severity using disease progress curve. Plant disease quantification is necessary to understand the disease impact on crop yield, improve breeding programs for

developing disease resistance, evaluate disease management strategies, understand the coevolution of plant and pathogen populations, and to study pathogen ecology and disease epidemiology (Bock et al. 2010 2016; Madden et al. 2007). Inaccurate plant disease assessment can lead to the failure of disease management strategies. For a practical disease management approach, it is necessary to quantify pathogens associated with its host and environment.

Phytopathometry can be performed either with traditional methods or with molecular methods. In the past years, pathogens detection was carried out through conventional methods that rely on morphological, microscopic, and cultural approaches (Nilsson et al. 2011). These approaches require labor, skill, extensive time, and previous knowledge of a pathogen. In the morphological approach, disease-causing agents are identified through a skilled observer's visual examination of the infected tissues (Chalupová et al. 2014). But in some cases, symptoms cannot be observed visually, resulting in pathogen identification complications. This approach is not sensitive and specific enough to identify the pathogen. It is also not suitable for regular analysis of a large number of pathogens. Low reproducibility in pathogen detection, lack of phylogenetic information, and stressed or injured pathogens are some major drawbacks of the cultural approach. These stressed pathogens do not show visible colonies on culture media, thus resulting in difficulty in identifying pathogens (Oliver 2005). Due to these limitations in the conventional diagnosis of phytopathogens, molecular approaches have revolutionized the pathogen identification, classification, and characterization in plant pathology.

These techniques include PCR-based detection methods based on genotypic characters rather than on morphological observations as in conventional methods (Spring and Thines 2010; Badali and Nabili 2012). These methods are more reliable, rapid, highly sensitive, and specific with no need for prior knowledge of classical taxonomy. Also, the isolation and purification of pathogens do not require in vitro culturing. Other molecular techniques are DNA or RNA probe-based methods (northern blotting, in situ hybridization, and fluorescence in situ hybridization), post-amplification techniques (DNA microarray and DNA macroarray), Isothermal amplification-based methods (rolling circle amplification, loop-mediated isothermal amplification, and nucleic acid sequence-based amplification), RNA interference, next-generation sequencing (DNA-Seq-based NGS and RNA-Seq-Based NGS), and DNA barcoding (Capote et al. 2012; Aslam et al. 2017).

10.2 PCR-Based Detection Methods

Nucleic acid-based diagnostic methods are DNA, RNA, and protein-based detection techniques. Through different primers and probes, many nucleic acid-based assays have been designed. These primers bind to a targeted organism's specific DNA or RNA sequence, which will be highly specific to a population's taxon (genus, species, or strain) (Aslam et al. 2017).

10.2.1 Conventional Polymerase Chain Reaction

Conventional PCR (conventional polymerase chain reaction), along with its variants, is one of the most common DNA-based detection methods adopted by many molecular laboratories for accurately identifying, quantifying, and characterizing plant pathogens (Baldi and La Porta 2020). An American biochemist Kary Banks Mullis invented the PCR technique in 1983 (Awarded with a Nobel prize in 1993), a great revolution in medicine, molecular biology, and plant pathology (Bartlett and Stirling 2003). This technique allows the synthesis (amplification) of a particular DNA sequence into multiple copies in no time. This can be achieved through repetitive cycles of three steps (denaturation, annealing, and elongation) at different temperatures. All the reaction mixtures such as DNA, primers or probes (oligonucleotides), dNTPs (deoxyribonucleotide triphosphates), buffer, and a *Taq Polymerase* (thermostable enzyme), are mixed in a tube and placed in a PCR machine. Primers are short nucleotide sequences that bind to a specific region of DNA by complementary base pairing (Mullis and Faloona 1987; McCartney et al. 2003). Specific amplification of target-specific nucleic acid sequence is used to identify and detect the disease-causing pathogen in plants. After the PCR reaction, the amplified DNA product is visualized using gel electrophoresis on agarose gel, stained with ethidium bromide. The presence of a specific DNA band in gel electrophoresis shows the confirmation of a specific disease-causing agent. PCR was primarily used to detect bacteria and viruses (Cai et al. 2014). But now, it is also used for the detection of fungal phytopathogens.

PCR is a widely used technique due to its precise, sensitive, rapid, and simplest nature because the detection is possible from a complex mixture and a single spore. It also has the potential to detect non-culturable pathogens such as RNA-based viruses and some bacteria, including phytoplasma (Henson and French 1993; McCartney et al. 2003). PCR performance depends on dNTPs (deoxyribonucleotide triphosphate) concentration and the efficiency of DNA extraction, polymerase type, activity, cycling parameters, and the stability and composition of the buffer. Its efficacy may also be affected due to the presence of inhibitors in the sample (López et al. 2006; Fang and Ramasamy 2015). To overcome the PCR inhibitors effect, CTAB (cetyltrimethylammonium bromide), along with specific enzymatic and chemical treatments, is considered the most helpful method for DNA isolation. To recover the DNA during isolation, silica matrix purification is preferable over ethanol precipitation during the phenol-chloroform step (Mancini et al. 2016).

Although PCR is a highly specific technique and provides accurate results, it is costly, requires too much labor, and involves a series of steps (sampling, genomic DNA isolation, primer designing, gel electrophoresis, DNA purification, etc.). Additionally, to start the process of DNA replication, there is a need to design sequence-specific primers, which is problematic for the samples collected from the field. Sometimes, the accurate results are not achieved by a single primer pair, so various other PCR variants are now widely used over the traditional PCR. In 1991, the PCR technique was firstly used for plant disease diagnosis to identify and detect a bacterium, *Pseudomonas syringae pv pisi*, also known as pea bacterial blight

(Rasmussen and Wulff 1991). Henson and French (1993) also identified several other fungal pathogens such as *Mycosphaerella fijiensis* (causing leaf spot disease in plantain) and *M. musicola* (causing leaf spot disease in a banana) through PCR technology. Two fungal pathogens, *Sclerotium rolfsii* (in 2010) and *Colletotrichum capsici* (in 2011), have been identified through PCR technology based on a specific sequence of ITS region (Torres-Calzada et al. 2011; Jeeva et al. 2010).

10.2.2 End-point PCR

PCR discovery has revolutionized the molecular and plant pathology fields for precisely identifying and detecting phytopathogens, including fungi. End-point PCR is commonly used to detect the presence or absence of DNA/RNA targets from the environmental samples and to check the relative quantity of targets (Thalinger et al. 2021). Specific primers and universal primers are designed (for amplifying specific fungal pathogens or multiple pathogenic species, respectively) in end-point PCR for specific detection of fungal phytopathogens. The identity for each set of nucleotide sequences can be examined by comparing the fungal isolates with ex-type fungal cultures present in NCBI (National Centre for Biotechnology Information) GenBank database using BLAST software. The targeted DNA/RNA in agarose gel ensures the presence of specific plant pathogenic fungi in the sample (Moher et al. 2015).

End-point PCR technique is more economical and user-friendly than other PCR-based assays for fungal phytopathogens isolation and detection. But it is also laborious and challenging to construct primer pairs to delimit the closely linked pathogenic fungal species. Two fungal species, *Phacidiopycnis washingtonensis* (causing speck rot in apple) and *Sphaeropsis pyriputrescens* (causing apple rot diseases), are identified and diagnosed by end-point and quantitative PCR assays (Sikdar et al. 2014). For quick diagnosis and detection, the qPCR (quantitative or real-time) assay was considered more sensitive and precise than end-point PCR. Several other plant pathogenic fungi have been diagnosed and characterized through the end-point PCR method. These included *Neopestalotiopsis clavispora* and *Colletotrichum siamense*, causing leaf spot disease in Macadamia (Prasannath et al. 2020), *Golovinomyces cichoracearum*, responsible for Hemp powdery mildew disease (Pépin et al. 2018), *Exobasidium maculosum*, causing Blueberry leaf and fruit spot (Brewer et al. 2014) and *Cercospora tezpurensis*, causing leaf spot disease in *Capsicum assamicum* (Meghvansi et al. 2013; Babar et al. 2021), *Curvularia lunata* in *Chamaedorea seifrizii* and *Trifolium alexandrinum* (Haq et al. 2021; Haq et al. 2021), *C. buchloas* in *Medicago sativa* (Haq et al. 2021), *C. tuberculata* in *Archontophoenix alexandrae* (Haq et al. 2020a, b, c), and *Alternaria alstroemeriae* in *Caryota mitis* L. (Haq et al. 2020a, b, c).

10.2.3 Bio-PCR

Bio-PCR is a modified variant of the end-point PCR technique that has an extra step of pre-assay incubation to enhance the number of pathogens for easy detection in contaminated samples. It is the combination of biological and enzymatic amplification of PCR targets. Bio-PCR mainly focuses on targeted pathogens by increasing their number on the growth medium and inhibiting the growth of non-targeted pathogens for improved sensitive detection. Bio-PCR has several advantages over conventional and other PCR-based methods that it exhibits a higher level of sensitivity, DNA extraction is not required before amplification, and elimination of false-positive and negative results because of the dead cells found in the seed also extracts due to the presence of PCR inhibitors in seeds, respectively (Schaad et al. 1995; Marcinkowska 2002). However, it is a highly effective technique for the sensitive detection of seed-borne fungal phytopathogens. However, it is costly and more time-consuming than other PCR-based assays when selective media is required in Bio-PCR (Mancini et al. 2016).

A fungal phytopathogen, *Colletotrichum lupini*, is responsible for seed and airborne lupin anthracnose disease, resulting in significant yield loss and mainly affects the stems and pods also identified and diagnosed with the help of Bio-PCR assay. Three important species of seed-borne fungal phytopathogens including *Alternaria radicina*, *A. alternata*, and *A. dauci* infecting carrot, are identified and detected by developing specific primers based on ITS (Internal Transcribed Spacer) region in rDNA during the Bio-PCR method along with DFBM (deep-freezer-blotter) (Konstantinova et al. 2002). In this method, seeds were incubated in amended Yeast Malt Broth to enhance the biomass of *C. lupine,* and the detection was done by using species-specific primers of IGS (Intergenic spacer) sequence in rDNA in lupin species (Pecchia et al. 2019).

10.2.4 Nested PCR (N-PCR)

Nested PCR is a modification of end-point and traditional PCR when an improvement in specificity and sensitivity of a sample is required. It is also 1000 times more sensitive than conventional PCR (Yeo and Wong 2002). Its potential has been reported in plant pathology as many infected viruses and bacteria have been identified in plants (Roberts et al. 1996; Olmos et al. 1997). Various PCR variants have been developed with higher specificity and sensitivity that permits the quantification of many infected pathogens in plants. In this PCR variant, two sequential rounds of PCR amplification reactions are performed, which use two pairs of primer. A single external primer pair is used for generic amplification, and its product is considered a template for the second cycle of PCR amplification via internal primer pair (Porter-Jordan et al. 1990).

Before a nested PCR is carried out, the products from the first PCR round are transferred to a separate tube with one internal primer (heminested amplification) or two internal primers (nested amplification). As the two reactions are to be performed

in two different tubes, this approach is more prone to cross-contamination, leading to an increased risk of false-positive results, particularly when this variant is used on a large scale daily. In addition, using two primer pairs makes this technique more inefficient and affluent than traditional PCR because of two PCR amplification reactions (Rahman et al. 2013). Another drawback is that there is an interference between two primers during amplification. For this, there is a need to precisely maintain the external and internal primer ratio and the minimum amount of external primers required to reduce intrusion during the second PCR amplification reaction.

The chances of cross-contamination risks can be avoided if laboratories follow strict precautions for each PCR cycle to be performed in a different place with separate equipment (Trtkova and Raclavsky 2006). Moreover, some researchers proposed that by improving the relative concentration of internal and external primers, a single-tube nested PCR allows high-throughput can be used to eliminate the drawbacks of this approach. It is a method that identifies and detects some viruses (Yourno 1992) and numerous bacteria such as *Erwinia amylovora* and *Pseudomonas savastanoi* (Llop et al. 2000; Bertolini et al. 2003) and fungal pathogens. Several nested PCR protocols have been developed for rapid and highly sensitive identification and detection of phytoplasmas (Khan et al. 2006; Samuitiene and Navalinskiene 2006). Nested PCR and printed or squashed samples on nitrocellulose membrane are used to detect RNA targets from many plant viruses and individual insect vectors (Moreno et al. 2007). Two Phytophthora species (P. palmivora and P. parasitica), responsible for Orchid disease, were identified using nested PCR (Tsai et al. 2006). A fungus, Phytophthora cactorum, a causal agent of leather rot in strawberries is also detected through nested PCR (Bhat and Browne 2010).

10.2.5 Co-operational PCR (Co-PCR)

A new PCR variant, co-operational PCR (Co-PCR), was developed to detect plant viruses and bacteria. It is carried out in a simple reaction with the simultaneous action of four primers, one primer pair external to the other, resulting in four amplicon production. And the co-operational action of these amplicons leads to the production of the longest fragment. It has minimum chances of contamination and low risk of manipulation with increased specificity and sensitivity, and also uses a small volume of reagents ten times less than traditional and nested. As the small volume of reagents could enhance the susceptibility toward inhibitors, a large quantity of RNA is required to obtain good sensitivity for detection (Olmos et al. 2002, 2005; Caruso et al. 2003). In both Co-PCR and nested PCR, the external primer pair is used to amplify a large sequence of targeted DNA while the internal primer pair, for more specific detection and characterization of amplicon obtained from the first PCR reaction at species and strain level (Capote et al. 2012).

Co-PCR was firstly developed for the successful sensitive detection of plant RNA viruses, including CTV (Citrus tristeza virus), CLRV (Cherry leaf roll virus), CMV (Cucumber mosaic virus), SLRSV (Strawberry latent ringspot virus), and PPV (Plum pox virus) (Olmos et al. 2002) and for the identification of bacteria, *Ralstonia*

solanacearum, in water and in cuttings of *Pelargonium* species (Caruso et al. 2003; Marco-Noales et al. 2008). Coupling of Co-PCR and dot blot hybridization by using a particular probe and with colorimetric visualization is used to allow the characterization of nucleotide sequence. The sensitivity and specificity level for viral detection is 1000 times more than the conventional and RT-PCR and similar to nested RT-PCR, which is used for bacterial detection. Grapevine fungi (*Phaeomoniella chlamydospora*) have been identified and characterized by combining co-PCR with dot blot hybridization and the colorimetric detection method (Martos et al. 2011). There are also some other protocols of Co-PCR for detecting plant pathogens such as *Candidatus Phytoplasma pyri*, *Candidatus Phytoplasma prunorum,* and *Candidatus Phytoplasma mali* (Bertolini et al. 2007).

10.2.6 Multiplex PCR (M-PCR)

Another PCR variant, multiplex PCR, was designed for the simultaneous sensitive detection of multiple targeted DNA or RNA sequences in the same reaction mixture, involving different PCR primer pairs for every target, hence the time and money can be saved without compromising on the efficiency (James 1999; López et al. 2009). It is considered a valuable tool and has a great potential in plant pathology where several phytopathogens (viruses, fungi, or bacteria) infest a single host plant; thus, simultaneous and specific detection is required (López et al. 2006). In M-PCR, more than one DNA fragment is simultaneously amplified that is specific to the targeted infecting pathogen. These fragments can be detected and visualized based on the molecular weight on the agarose gel. DNA amplification efficiency is strongly affected because of the amplicon size, as a shorter amplicon amplifies more rapidly than a larger amplicon. To overcome this problem, the accurate design of primers is necessary with the optimized condition of their relative concentration and annealing temperature so that all the amplicons must be generated and detected efficiently (Dasmahapatra and Mallet 2006).

Many researchers have used this variant for the simultaneous detection and identification of various phytopathogens such as *Phytophthora lateralis* in water and cedar trees (Winton and Hansen 2001), *Tapesia acuformis* and *T. yallundae* (mating type pathogens) (Dyer et al. 2001), for detection and differentiation among 11 taxons of wood rotting fungi infesting different hardwood trees (Guglielmo et al. 2007), for distinguishing two powdery mildews *Golovinomyces cichoracearum*, *Podosphaera xanthii* and *Macrophomina phaseolina* in sunflower (Chen et al. 2008; Ijaz et al. 2013), for the simultaneous identification of plant pathogenic fungi (*Fusarium oxysporum*, *Phytophthora nicotianae*, *Phytophthora cactorum*, as well as *Bipolaris cactivora*) that are responsible for infection in grafted Cacti (Ji Cho et al. 2016). However, few examples are found where three or above three plant viruses are amplified in M-PCR due to the presence of different compatible primers in a reaction mixture. Two examples include the simultaneous detection and differentiation of six olive tree viruses (SLRSV, CMV, Olive latent virus-1 (OLV-1), Olive latent virus-2 (OLV-2), CLRV, and Arabic mosaic virus) by using twenty

compatible primers (Bertolini et al. 2001) and the simultaneous detection of nine viruses including grapevine virus A, grapevine virus B, grapevine fleck virus, grapevine leafroll-linked virus-1, -2, -3, ArMV, rupestris stem pitting-linked virus, and grapevine fleck virus, causing disease in grapevine (Gambino and Gribaudo 2006).

Currently, PLPs (padlock probes) from the LD (ligation detection) system are used in the multiplex PCR approach to identify and simultaneously detect plant pathogenic fungi and oomycetes. Padlock probes are long oligonucleotide-based probes with compatible sequences at their 5′ and 3′ ends to target DNA. PLPs include ZipCode (unique sequence identifier) for standard microarray hybridization, an internal endonuclease IV (cleavage site for linearization), and a desthiobiotin moiety to capture and release. After the DNA sample is amplified, it is subjected to PLP ligation. Upon hybridization of PLPs to the targeted DNA, they are circularized by an enzyme ligase. After that, probes are captured on streptavidin attached with magnetic beads and cleave at the internal site. So, the original ligated PLPs will be detected through ZipCode microarray. Several phytopathogens have been simultaneously identified and detected by PLPs in complex samples from horticulture water circulation systems. These pathogenic microorganisms are Pythium undulatum, P. aphanidermatum, P. ultimum, Fusarium solani, F. oxysporum, Phytophthora nicotianae, P. cactorum, Verticillium albo-atrum, V. dahlia, Myrothecium verrucaria and M. roridum (van Doorn et al. 2009). Several other multiplexing tools have replaced m-PCR variants like multiplex nested PCR and multiplex RT-PCR.

10.2.6.1 Multiplex RT-PCR

The sequence of different pairs of compatible primers, specific to multiple targets, must be checked in silico and trials must be performed in a laboratory, so multiplex RT-PCR is designed. As in simple M-PCR, general and ordinary primers, those that are based upon 16SrRNA gene sequence, are not suitable for the amplification of multiple DNA targets. The reason is that multiple targets are competing with each other by making the reaction more detectable for the most abundant targets and difficult for less abundant targets. In real-time multiplex PCR, more than two probes that are different from each other are required for simultaneous detection.

10.2.6.2 Multiplex Nested PCR

Multiplex nested PCR combines both multiplex and nested PCR with enhanced sensitivity and reliability, reducing the reagent cost and time as the two PCR reactions are taking place simultaneously in a single PCR machine. This variant is suitable for simultaneously detecting multiple DNA/RNA targets by using separate primers for each target. During primer designing, care is needed to avoid primer dimer and hairpin formation. Some researchers have coupled the multiplex nested PCR with colorimetric visualization to enhance the interpretation of results and sensitivity.

10.2.7 Reverse Transcription PCR (RT-PCR)

Several changes have been made in PCR procedure since it was discovered. Some changes in procedure have expanded the diagnostic capability and use of this technique in many medical and biological fields. Other variants of PCR cannot differentiate among dead or living fungi and its structure, so the development of the RT-PCR variant is necessary to overcome this limitation. Several pathogenicity tests must evaluate the results obtained from the identification and detection of plant fungi. Although messenger RNA (mRNA) is rapidly destroyed in dead cells, detecting mRNA through RT-PCR is important to check the viability of cells (Capote et al. 2012; Sheridan et al. 1998). In this method, mRNA is firstly reverse transcribed into complementary DNA (cDNA) with the help of general primers and an enzyme called RT (reverse transcriptase). This cDNA is then amplified through any traditional or another PCR method.

Through RT-PCR, many RNA-containing viruses (retroviruses) have been identified and detected, important for synthesizing and estimating the effectiveness of antibacterial vaccine therapy. It is also used for rapid detection of a viable population of fungus, *Mycosphaerella graminicola*, in wheat crops (Guo et al. 2005). Nested PCR coupled with RT-PCR (RT-nested PCR) was used to detect and diagnose a fungal pathogen, *Oidium neolycopersici*, in tomato (Matsuda et al. 2005). In many cereal crops like wheat, maize, barley, rye, and oat, a fungus (*Fusarium graminearum*) responsible for Fusarium ear blight disease is also detected and quantified by the RT-PCR approach (Brown et al. 2011). Moreover, this approach is also useful for gene expression analysis of plants (susceptible barley) and fungi (Fusarium graminearum) during the early development of disease (Yang et al. 2010).

10.2.8 Magnetic Capture Hybridization PCR (MCH-PCR)

The magnetic capture hybridization PCR (MCH-PCR) method was established to overcome the effects of PCR inhibitor molecules in plant tissues. It is based on the DNA extraction method involving a purification step in which hybridization takes place by using a single-stranded DNA probe on the magnetic beads that is highly specific to the targeted DNA sequence and amplifies it (Jacobsen 1995). The process of hybridization occurs between the targeted fungal DNA and magnetic beads coated with biotinylated oligomers, separating a compound from the inhibitors (Capote et al. 2012). Once the magnetic capture hybridization is done, PCR amplification is performed via species-specific primers. An oligonucleotide sequence-based probe in MCH-PCR is designed by selecting the highly conserved regions of fungal phytopathogens along with the specificity of the targeted sequence is checked by performing BLAST. It was used to detect and evaluate a fungus, *Nectria galligena*, causing an infection to pear and apple trees (Langrell and Barbara 2001). MCH-PCR and real-time PCR assays were used to detect and diagnose two seed pathogens of cucurbit seed samples, including *Didymella bryoniae* (a fungus responsible for

gummy stem blight) and *Acidovorax avenae* (cause bacterial fruit blotch) (Ha et al. 2009). It increases PCR sensitivity, decreases detection time, and eliminates the effects of PCR inhibitors and an excess of non-target DNA (Amagliani et al. 2006).

10.2.9 PCR-ELISA

Polymerase chain reaction-enzyme linked immunosorbent assay (PCR-ELISA) is a merge tool of both PCR and ELISA, making it a powerful tool for detection. It is based on an immunological method to detect and quantify the PCR amplicons. This method involves two primers (forward and reverse) labeled with biotin and an antigenic group (fluorescein) separately at their 5′ ends (Landgraf et al. 1991). An amplified PCR product is then immobilized on the microtiter plates, and this is achieved due to the presence of avidin or streptavidin on microplates and biotin of a forward primer. However, the quantification is done by an ELISA with a colorimetric detection signal because an antigenic group is present in the reverse primer. It is highly sensitive and precise like nested PCR and has a lower detection limit and analytical time. Another notable advantage of this technique is that it can be easily handled, error-free and there is no need for a hybridization process or electrophoretic separation. PCR-ELISA is carried out in microtiter plates with 96 wells to quantify PCR products for routinely diagnostic purposes. This assay has been used to detect and differentiate a fungus, *Didymella bryoniae*, in cucurbits and numerous species of Pythium and Phytophthora (Somai et al. 2002; Bailey et al. 2002).

10.2.10 In situ PCR

In situ PCR is a new molecular approach that combines the benefits of both PCR for increased sensitivity and in situ hybridization for cellular localization. It is a useful technique as it amplifies particular sequences directly within integral plant cells or tissues (Long 1998; Nuovo 1994). This technique works less efficiently due to high background detection because nonspecific DNA synthesis may occur on tissue segments (Nuovo et al. 1994). Also, it is a laborious technology because demanding an extra step of hybridization and protocols for other techniques like light microscopy. In situ PCR was used to identify and detect spores as well as mycelia of *Blumeria graminis* fungus on the leaves of barley (Bindslev et al. 2002).

10.2.11 PCR-DGGE

PCR-DGGE (Polymerase chain reaction denaturing gradient gel electrophoresis) is a new variant of PCR that allows the genetic diversity analysis of microbial ecosystems and monitors the population dynamics without requiring previous knowledge of species (Portillo et al. 2011). When DNA samples are running in an acrylamide gel, they are separated via denaturing gradient gel electrophoresis

(DGGE) or its variant temperature gradient gel electrophoresis (TGGE) by using a chemical gradient (urea) or temperature, respectively. In the PCR-DGGE technique, PCR amplification is done for the targeted DNA samples obtained from the environment or plant, followed by denaturing electrophoresis. Denaturing gradient gel employs the separation of PCR products, and DNA sequence variants of specific fragment lengths move at different levels, so it is a powerful technique, allowing highly effective in the identification of polymorphisms in several targeted DNA sequences. Also, specific primers are designed for PCR-DGGE, which are rich in GC content at the 5′ end to increase the detection capability.

The visible and well-separated bands in the gel can be isolated, re-amplified, or cloned and subjected to sequence and identification. Even identifying elements that show up to 1% of the whole microbial community is possible through PCR-DGGE technology. Furthermore, this technique is helpful for rapidly identifying numerous microbial species and for detecting unknown or novel microbes. However, this technique has several drawbacks, such as giving the comparative information for the abundance of identified species, which is more time-consuming with poor reproducibility. The presence of microheterogeneity in specific targeted genes may cause the various bands to appear in a gel for single species resulted in overestimation of community genetic variation, and hence there may be a difficulty in results interpretation. Also, DNA sequence variants with different fragment lengths but with the same melting temperatures are not always appropriately separated.

In some scenarios, the genetic diversity study of complex microbial communities and various plants such as shisham could provide unclear gels because many bands are found during PCR-DGGE (Capote et al. 2012; Ijaz et al. 2018). This method helps to detect and compare the structure of various fungal communities, including Phytophthora species present in different environmental samples or plant materials. For example, the structure and relative abundance of soil microbial and fungal communities are studied by the cultural management practices like chemical fumigation, biofumigation, or fertilization (Wakelin et al. 2008; Omirou et al. 2011).

10.2.12 Real-Time or Quantitative PCR (qPCR)

Traditional PCR only amplifies targeted DNA, which is insufficient for rapid detection and diagnosis of diseases. In the past few years, advanced technology has been introduced in plant pathology to detect phytopathogens and is considered modified version of traditional PCR, where targeted DNA or RNA is amplified and quantified in a PCR reaction mixture in real-time (Mackay 2004). This technique's monitoring of the reaction during PCR amplification is possible because of the fluorescent dyes like SYBR Green I or *Taq* Manprobe (fluorescent-labeled probes based on specific sequences), Molecular Beacons, Eva Green, dye-based primer systems like hairpin primers or Plexor system. The fluorescent signals are produced when dyes intercalate to DNA or RNA targets, and thus, signal intensity is based on how many targets are present within the samples and the signal upsurges as the number of generated amplicons upgrades after each cycle of PCR amplification

(Wittwer et al. 1997; McCartney et al. 2003; Badali and Nabili 2012). Sequence-specific primers are important to determine the relative copy number of targeted DNA/RNA sequences by projecting a Ct (cycle threshold) value (Balodi et al. 2017).

qPCR has various benefits that overcome the drawbacks of traditional PCR in terms of using less time, fewer reagents, cost-effective, reducing labor assay, fast and more accurate, high specificity along with improved sensitivity, and there is no need for post PCR processing methods (e.g., electrophoresis, hybridization, and colorimetric reactions), prevent the chances of cross-contamination. The improved sensitivity is important in plant pathology for estimating fungal pathogens in a plant sample to check the disease condition and the level of disease in a damaged sample (Garrido et al. 2009). Additionally, during detection, it allows the detection of other variants of pathogens, point mutations in genes, and quantification of originally targeted pathogens. This technique also has certain limitations, such as the fluorescent dye for monitoring purposes is non-specific. The intercalating dye binds to all the target DNA within a sample, causing primer dimer formation; hence, false results are obtained. So fluorogenic probes are preferable to fluorescent dyes because of their high efficiency (Bu et al. 2005; Atkins and Clark 2004). Two fluorescent dyes, such as reporter dye and quencher dye, are linked with these probes to the 5′ end and 3′ end, respectively, and the close proximity of these two dyes prevents the fluorescence emission. As the PCR reaction proceeds, reporter dye separates from quenching dye because of the exonuclease activity of *Taq* Polymerase enzyme, and reporter dye produces light signals (Dasmahapatra and Mallet 2006).

The quantitative PCR has been proposed to detect and quantify various infectious fungal pathogens, including *Cladosporium cladosporioides*, *Alternaria alternate*, *Aspergillus versicolor*, *Stachybotrys chartarum,* etc. (Black 2009). An emerging and hypervirulent plant fungus, *Cryphonectria parasitica*, is responsible for several diseases such as wilting, lethal bark canker, blight, and dieback in three plant species (Chestnut trees, *Cannabis sativa,* and *Castanea dentate*) (Jain et al. 2019; Murolo et al. 2018) is also detected and quantified through qPCR by utilizing rDNA ITS regions with a precision of approximately 2 fg of genomic DNA sequences that was equal to the fungal pathogen single spore (Chandelier et al. 2019). A qPCR protocol was developed to distinguish and quantify several fungal phytopathogens such as *Ramularia collo-cygni*, which cause small and brownish spots on leaves, awns, and sheaths in seeds of barley (Havis et al. 2014), *Verticillium longisporum* (Depotter et al. 2017), *Diaporthe gulyae* and *Diaporthe helianthi*, causative agents of phomopsis stem canker in sunflower (Elverson et al. 2020), and Neopestalotiopsis species in guava (Haq and Ijaz 2019). Fusarium solani complex and Colletotrichum spp., causative agent of dieback disease were also detected and quantified through qPCR (Haq and Ijaz 2019; Latif et al. 2021; Yasin et al. 2022; Iqbal et al. 2018).

10.2.13 Droplet Digital PCR (ddPCR)

Droplet digital PCR (ddPCR) is the latest technology that enables the exact quantification of nucleic acid targets in a sample. The sample containing the targeted DNA is

fractioned into about 20,000 nanoliter-sized droplets, and PCR reaction of the template molecule is performed independently in each droplet. So fluorescence measurement estimates the exact quantity of amplified target DNA for every droplet (Hindson et al. 2011). This technology presents enormous benefits over traditional and real-time quantification PCR, such as unparalleled sensitivity when detecting low copy number genes, absolute quantification with no need for a standard curve, high precision, and through different fluorescence signals, multiple targeted genes in a single PCR reaction can be identified (Hayden et al. 2013). Additionally, it is highly resistant to PCR contaminants that can cause major problems during the analysis of plant samples (Blaya et al. 2016; Rački et al. 2014). However, ddPCR has not been recognized for POC study in agriculture so far but some methodologies of ddPCR have been developed for the quantitative detection of different viruses and fungal pathogens (Liu et al. 2019; Selvaraj et al. 2018; Zhong et al. 2018). While it was planned to be used in clean plant programs nationally to avoid the importation of pathogen-infected nursery stock (Voegel and Nelson 2018).

10.3 DNA or RNA Probe-Based Methods

DNA/RNA probe methods are highly sensitive, cost-effective, and require less time to identify pathogens, particularly fungi. These techniques include northern blotting, in situ hybridization, and fluorescence in situ hybridization (FISH). These techniques use the probe for DNA/RNA analysis with no need for its amplification. The probe is a short, single-stranded DNA or RNA sequence that is radioactively or fluorescently labeled. These probes identify the homologous sequences present in the targeted gene. In the traditional method, these probes are used as a replacement for PCR but in novel techniques, these are used in combination with PCR for effective pathogen detection (McCartney et al. 2003).

10.3.1 Northern Blotting

Northern blotting is an RNA-based technique to evaluate the gene expression of pathogens. In Northern blot (named as RNA blot), RNA is transferred to a carrier to identify the targeted pathogen and the level of disease caused by this pathogen. The northern blot is just like the Southern blot, with the only difference being the use of RNA rather than DNA. In Northern blotting, RNA is purified from a homogenized tissue sample to examine the expression of the targeted gene. A labeled probe binds to its complementary sequence (Kim et al. 2010). In autoradiography, a visible band should be detected if a probe hybridizes to its complementary RNA sequence. RNA size can be estimated from the position of a band on the blot. The main drawback of this technique is the possible degradation of RNA in gel electrophoresis. Secondly, multiple probes give problematic results for pathogen detection. The sensitivity of this technique is comparatively less to RT-PCR. *Magnaporthe grisea* is a fungal

pathogen that has been detected in rice crops using northern blotting and RT-PCR (Qi and Yang 2002).

10.3.2 In Situ Hybridization

In situ hybridization (ISH), also known as hybridization histochemistry, is a molecular technique for pathogen detection. In this method, a single strand (ss) radioactive probe is used, also known as a riboprobe that binds to its complementary RNA sequences of a targeted pathogen. These ssRNA probes are categorized with radioactive isotopes, ^{35}S, ^{32}P, and ^{125}I that give efficient and most sensitive results (Hayden et al. 2002). It is similar to northern blotting, with the only difference of starting material that is a histological section rather than a tissue digest (Jensen 2014). The main hindrance of this technique is that radiolabeled probes are hazardous and quite expensive. These probes must be handled and disposed of with intensive care. Another drawback of this technique is that low RNA/DNA concentrations cannot be detected (Qian and Lloyd 2003). This tool has been used to identify various fungal pathogens such as *Sporothrix schencki, Blastomyces dermatitidis, Cryptococcus neoformans, Histoplasma capsulatum*, *Puccinia horiana*, and *Coccidioides immitis* (Hayden et al. 2001).

10.3.3 Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) is a recent and advanced molecular technique for rapidly characterizing pathogens such as fungi, bacteria, and viruses. High sensitivity, reproducibility, accuracy, safety, specificity, and swiftness are the paramount attributes of FISH (Bozorg-Ghalati et al. 2019). It comprises a high potential to provide details about specific pathogen identification, morphology as well as resolution from a combination of pathogens (Frickmann et al. 2017). A fluorescent probe is used to identify a targeted sequence found in the pathogen genome. It binds to only that chromosome sequence with which it has a high level of complementary and homology. The targeted sequences of the probe are either ribosomal RNA or mitochondrial genome. It works by direct hybridization of fluorescently labeled oligonucleotide probe with mRNA of targeted phytopathogens. Then these hybridized sites are detected and visualized through confocal laser scanning microscopy or wide-field epifluorescence microscopy (Lukumbuzya et al. 2019). The fluorescent probes are developed through enzymatic integration of fluorophore-modified base. Various probes that can be used in this technique are a centromeric repeat-based probe, locus-specific probe, and whole chromosome-based probe (Volpi and Bridger 2008).

In case of traditional techniques for pathogen detection, cells need to be in a vigorously and actively dividing stage. Conversely, FISH has the potential to identify non-dividing cells, which makes it an extremely useful and adaptable technique. These non-dividing cells could be identified even with a low degree of

fluorescence strength. *Sclerotium rolfsii*, a soil-borne pathogen that causes potato blight disease in tomato was detected by FISH using cyanine-labeled probes (Cy3 and Cy5 cyanine dyes) (Milner et al. 2019). However, some pitfalls can limit its application for phytopathogens detection. In FISH, the process of fluorochrome-labeled probe development is quite complicated (Volpi and Bridger 2008) and results in fast positive outcomes because of these autofluorescence materials. The precision and reliability of FISH assay mainly depend on the specificity of the fluorescent probes. Insufficient hybridization, high order secondary structures (RNA-protein interaction, 3-D RNA structure, and formation of loop and hairpin structure) in the probe and its target sequence, low ribosome content, and photobleaching could also give false-negative results (Moter and Göbel 2000).

10.4 Post-amplification technique

10.4.1 Macroarray

Macroarray is also acknowledged as DNA array hybridization or else Reverse Dot Blot Hybridization (RDBH) (Singh and Kumar 2013). This assay is based on the hybridization of amplified and labeled targeted gene to immobilized probes (species-specific 15–30 bp oligonucleotides) dotted on a fixed support platform (nylon or nitrocellulose membrane) by UV cross-linking (Leinberger et al. 2005; Tsui et al. 2011). It simultaneously analyses a larger number of PCR products (amplified targeted gene) in one hybridization reaction (Zhang et al. 2008). It is comparatively more sensitive and reliable than PCR. So, PCR amplification is incorporated with a hybridization reaction that results in 1000 folds increase in sensitivity compared with PCR alone (Taoufik et al. 2004). It requires less time (about 1–2 days) in comparison with the radioactive culture technique that requires 2–8 weeks for accomplishment. It was first established to detect mutation in the human genome. Now, this assay is becoming a widely used molecular tool for the identification of phytopathogens such as bacteria (Fessehaie et al. 2003), viruses (Agindotan and Perry 2007, 2008), nematodes (Uehara et al. 1999), and mainly fungi (Chen et al. 2009). It is quite an effective and reliable molecular assay where phytopathogens can be detected and identified from a mixture of pathogens without needing isolation and purification in culture (Tambong et al. 2006; Zhang et al. 2007, 2008).

Macroarray combined with PCR tool has been used to detect mycobacterium based on species level (Leinberger et al. 2005). Furthermore, macroarray was carried out to target species-specific *TUB2* gene sequence and then amplified by digoxigenin-labeled primers (Bt2a and Bt2b) in PCR. This array was then used to detect and discriminate 15 different fungal species from genera *Neonectria*, *Cylindrocarpon*, *Dactylonectria*, *Phaeomoniella*, *Cladophora*, *Ilyonectria*, and *Campylocarpum* as well as the black-foot disease-causing pathogens *Dactylonectria pauciseptata*, *D. macrodidyma*, *Ilyonectria robusta*, *I. liriodendra,* and *I. europaea* (Agusti-Brisach and Armengol 2013). 34 fungal pathogens that cause young wine decline (YWD) disease of young grapevine were also spotted through DNA

macroarray assay (Úrbez-Torres et al. 2015). It has also been used to detect and identify many fungal phytopathogens such as *Candida albicans*, *Alternaria alternata*, *Fusarium solani*, and *Cladosporium herbarum* (Sato et al. 2010). However, there are certain limitations due to which its sensitivity and reliability could be compromised. This array cannot quantify the targeted pathogen and also cannot give information about whether the isolated DNA is from a living pathogen (Úrbez-Torres et al. 2015).

10.4.2 DNA Microarray

A DNA microarray, also known as a gene chip, DNA chip, or biochip, is a collection of microscopic DNA spots fixed on a solid surface (commonly glass or nylon filters) in well-defined regions (Bhat and Rao 2020). These spots are comprised of 1000s of particular DNA sequences known as probes, hybridized with targeted cDNA from a sample. This hybridized probe-cDNA complex is then identified and quantified with silver, chemiluminescence, or fluorophore-labeled targets to evaluate the transcript level (cDNA quantification) in a sample using a software (Russo et al. 2003; Lievens and Thomma 2005). This software generates an expression profile for the targeted gene present in a sample (Russo et al. 2003). It permits the analysis of a wide range of mRNA and measurement of gene expression (Reymond 2001). Previously, it was used only to study gene expression, but new development has led to the identification of various phytopathogens such as fungi, bacteria, viruses, and viroids (Nam et al. 2014; Tiberini and Barba 2012; Krawczyk et al. 2017; Musser et al. 2014).

PCR primers along with fluorescent-labeled probes that target the genome of particular fungal potato pathogens, *Fusarium species* (*TEF- 1α*), *Alternaria alternate, A. solani*, (*Alt_ α1* gene), *Colletotrichum coccodes* (*TUB2*), *Rhizoctonia solani*, and *Spongospora subterranea* (ITS region) were used by RT-PCR microarray tool in 48-well silicon microarrays (Nikitin et al. 2018). *Aspergillus candida* species were also diagnosed using this technology (Singh and Kumar 2013). Although the DNA microarray technique is simple, reliable, fast, and cost-effective, there are some drawbacks. Firstly, high mRNA amount is required to assess expression level, physical cell disruption is necessary to evaluate gene expression profile, generation of false data due to mRNA degradation, and it can only detect sequences according to its deigned array (Bumgarner 2013). Recently, a new microarray technique (Arraytube) has been used to detect several root rot pathogens in beet, *Penicillium expansum*, *Botrytis cinerea*, and *Aphanomyces cochlioides* using specific marker gene sequences (*TEF-1α*, ITS, and 16S rDNA) along with operative probes (Liebe et al. 2016).

10.5 Isothermal Amplification-Based Methods

10.5.1 Rolling Circle Amplification

Rolling circle amplification (RCA) is a highly efficient isothermal amplification of circular DNA. It is an enzymatic assay that generates either RNA or single-stranded DNA (ssDNA) using DNA or RNA polymerase enzymes. DNA polymerase, short DNA/RNA primers, dNTPs (deoxynucleotide triphosphate), a circular DNA template, homologous buffer, DNA binding, and unwinding proteins are major perquisites for RCA assay (Gu et al. 2018). In RCA reaction, primers have dual roles, firstly as signal amplifier for RCA and secondly as a discriminator for their complementarity to the targeted sequence. Use of second primer, paired with the targeted sequence, could generate hyper-branched RCA (HBRCA) that can be utilized as a substitute of PCR for targeted gene amplification. In recent times, two fungal pathogens, *Cryptococcus gattii* and *C. neoformans*, are diagnosed using HBRCA assay (Trilles et al. 2014). This assay is based on the rolling replication of short ssDNA molecules.

Circularizing oligonucleotide probes (species-specific PLPs) are used in this assay. These probes are short and single-stranded, having target sites of 20 nucleotides at both ends (5′ and 3′) that are connected by a linker sequence of 40 nucleotides (Tsui et al. 2011). These probes bind to their targeted sequence resulting in the binding of both ends in circularized form using a ligase enzyme. These circularized probes span the targeted region's entire sequence due to the helical nature of dsDNA (double-stranded DNA). These probes can only detect that DNA fragment with known sequences (Wang and Yang 2009).

RCA is an efficient, simple, robust, and reliable technology for identifying and locating nucleic acid sequences (Gusev et al. 2001) with no need for temperature cycling devices (Goo and Kim 2016; Dong et al. 2013). Four podlock probes, PLP-Nv, PLP-Nm, PLP-Nk, and PLP-Np, were designed to target TEF-1α, resulting in the identification of *Neofabraea kienholzii*, *N. perennans, N. malicorticis*, and *N. vagabunda* that cause apple bull's eye rot disease. An RCA reaction was performed for the detection of these pathogens (Lin et al. 2018). In further analysis, an RCA reaction was conducted to diagnose and identify *Fusarium oxysporum*, *F. tricinctum*, and *F. graminearum* that cause Fusarium head blight disease. The padlock probes used in this assay were developed based on polymorphism in *TEF-1α* (Davari et al. 2012). This technique can also be used to study gene expression, mRNA splicing, single nucleotide polymorphism, and post-translational modification of proteins (Gao et al. 2019).

10.5.2 Loop-Mediated Isothermal Amplification

Loop-mediated amplification (LAMP) is a novel, robust, and specific DNA amplification technique which is a PCR alternative for diagnosing various plant diseases (Le and Vu 2017; Li et al. 2017). This technique is based on amplifying the targeted

sequences under isothermal conditions. Just like RCA, it also doesn't require any temperature cycling devices to generate thermal changes as an alternative, it only needs a single tube (single temperature) to amplify the targeted sequence (Tsui et al. 2011; Fakruddin 2011). Therefore, it is established on auto-cycling strand displacement activity for the targeted DNA amplification. The LAMP technique consists of two main steps: an initial stage with cycling amplification and an elongation stage (Panno et al. 2020). This assay uses a bst DNA polymerase enzyme and four sets of primers (two sets of internal primers and two sets of outer primers). Forward inner primer (FIP) and backward inner primer (BIP) are internal primers, while F3 and B3 are two outer primers that distinguish 6 unique sequences present on the targeted DNA sequences. Each FIR, as well as BIP primers, consists of unique sequences corresponding to the coding and non-coding sequences of targeted DNA. The addition of loop forward (LP) and loop backward (LB) primers can accelerate LAMP assay resulting in reduced time and increased sensitivity (Nagamine et al. 2002; Francois et al. 2011; Fakruddin 2011). LAMP reaction is performed in a water bath or heat block at 60–65 °C (an ideal temperature for bst DNA polymerase activity). Its high efficacy and exponential amplification yield 109–1010 times the target gene in just 1 h (Chander et al. 2014; Notomi et al. 2000). Later on, the resulting amplicon (amplification product) is spotted through SYBR Green dye or bromophenol blue dye in gel electrophoresis. These amplicons have several inverted repeats of the targeted gene that display a structure like cauliflower with many loops. LAMP technique is comparatively more precise, inexpensive, and reliable compared with traditional PCR (Ren et al. 2009; Waliullah et al. 2020).

However, laterally, LAMP was primarily developed for detecting DNA molecules; another modified technique, RT-LAMP, was established for targeted RNA detection and its amplification (Fukuta et al. 2013a, b). This technique has diagnosed various phytopathogens, such as fungi and viruses. Plum pox virus (PPV) is a plant virus that has been identified by this technique (Varga and James 2006). *Ascochyta rabiei* is a fungal pathogen causing ascochyta blight disease in chickpeas which has been identified using this method. For *A. rabiei* detection, LAMP, compared to conventional PCR, is not only an accurate, specific, and efficient method but also requires minimum operational time (Das et al. 2012). Despite its efficiency, there are major downsides involving indirect assessment methods such as SYBR Green dye, Mn^{2+} dye, hydroxynaphthol blue dye, and bromophenol blue dye that cannot distinguish the targeted sequence and nonspecific amplified products. Molecular beacons are fluorescent-labeled short oligonucleotide hybridization probes that can resolve this problem by generating fluorescent signal when it binds to their targeted DNA. So, LAMP products are directly assessed by these probes. Optimum requirements for molecular beacons are 25 bp to 45 bp beacon length, 0.6–1 pmol/μL concentration, and 60–65 °C temperature (Liu et al. 2017).

Paracoccidioides brasiliensis, *Penicillium marneffei*, *Ochroconis gallopava*, *Ophiostoma clavatum*, *Pythium aphanidermatum*, and *P. myriotylum* are various fungal pathogens that have been identified using LAMP method (Tsui et al. 2011; Fukuta et al. 2013a, b, 2014; Niessen 2015). *Uromyces betae* is a fungal pathogen that cause rust disease in sugar beet, was diagnosed via LAMP method within

30 minutes by targeting DNA sequence of cytochrome b protein (Kaczmarek et al. 2019). Another fungal pathogen, *Fusarium circinatum* that infects pine and conifers causing pitch canker disease was identified by conventional LAMP and quantitative LAMP methods. Hybridization of LAMP probes with *TEF-1α* sequence indicated that quantitative LAMP is more specific than cLAMP for detecting *F. circinatum* (Stehlíková et al. 2020). *Fusarium odoratissimum* tropical race 4 (TR4) that causes panama disease in bananas has been identified by LAMP tool using TR4 markers (Ordóñez et al. 2019). This method is highly specific and sensitive to using various primer up to six to amplify targeted nucleic acids (Becherer et al. 2020). Regardless of its efficiency, it shows some shortcomings due to compromised efficacy. Using six primers for a short DNA fragment, high amount of indicators and other constituents that can halt enzyme activity could exhibit some limitation using the LAMP tool (Tanner et al. 2015).

10.5.3 Nucleic Acid Sequence-Based Amplification (NASBA)

NASBA is a transcriptional and isothermal-based amplification method designed particularly for RNA detection of targeted phytopathogens. DNA can also be detected using some NASBA systems (Sergentet-Thevenot et al. 2008). In recent years, various DNA amplification methods have been developed, such as PCR, RCA, and LAMP, but none can amplify RNA directly. NASBA is preferable for mRNA amplification over other methods for its high sensitivity. This technique is based on RNA amplification followed by its conversion into complementary DNA (cDNA) through reverse transcriptase enzyme. It can produce $<10^8$ copies of nucleic acids (DNA/RNA) in just 1and 1/2 h incubation using three enzymes (Wernecke and Mullen 2014). It is an isothermal reaction carried out at 41 °C and hence doesn't need any thermal cycler (Loens et al. 2006), having high amplification power compared with RT-PCR (Chang et al. 2012). Three enzymes used in NASBA reaction are reverse transcriptase derived from avian myeloblastosis virus (for reverse transcription of RNA to generate cDNA), RNase H (for hydrolysis of RNA fragment from DNA/ RNA hybrid), and T7 RNA polymerase (for producing high copy number of ssRNA complementary to targeted RNA) (Chang et al. 2012).

An imperative feature of NASBA assay is the construction of ssRNA (NASBA amplicons) that could be used directly for additional amplification and inquired for further assessment without its degradation (Chang et al. 2012). NASBA products are then assessed via electrochemiluminescence or colorimetric detection using digoxigenin-labeled probes or via RT NASBA assay using molecular beacons (Heo et al. 2019). NASBA-beacon assay delivers results in less than 1 hour with less contamination risk because of RNA-based amplification. It quantifies phytopathogens RNA, particularly viruses, via mRNA expression (Fakruddin et al. 2012; Lauri and Mariani 2009). Several plant viruses have been detected using NASBA assays, such as Potato virus Y and Apple stem pitting virus (Klerks et al. 2001). *Clavibacter michiganensis* and *Ralstonia solanacearum* are two bacterial phytopathogens that have also been diagnosed by this tool (Schoen et al. 2005).

However, no fungal pathogen has been detected yet, but in the future, it shows great potential for the detection and quantification of fungal phytopathogens.

10.6 RNA Interference

RNA interference (RNAi) is the up-to-date tool for identifying and controlling phytopathogens, particularly mycotoxigenic fungi (Panwar et al. 2013). RNA interference depends on various factors such as constructing a vector that must generate double-stranded RNA followed by synthesis of small interfering RNA (siRNA), availability of targeted sites for RNAi in phytopathogens, high capability of phytopathogens to uptake siRNA, the half-life of siRNA, and amplification of the subsequent targeted gene. RNAi technology has intrinsic cellular defense activity for detecting targeted gene in the pathogen. This dsRNA or hairpin RNA (hpRNA) identifies their targeting homology sequences leading in the silencing or degeneration of that gene (Nakayashiki et al. 2005). For identifying fungal pathogens, specific hpRNA and dsRNA can limit fungal diseases. A major negative aspect of this tool is the off-target effect of these RNA molecules. This tool has been used in various plants to diagnose fungal pathogens such as *Fusarium graminearum* in barley (Chen et al. 2006) and *Aspergillus flavus* in Zea mays (Masanga et al. 2015).

10.7 Next-Generation Sequencing

NGS (next-generation sequencing), also recognized as high-throughput sequencing (HTS) is an advanced technology for diagnosing phytopathogens. This tool has presented various innovative methods for detecting and identifying pathogens (Chalupowicz et al. 2019). This approach is based on the sequencing of targeted DNA/RNA molecules. NGS can be either based on DNA-sequence-based next-generation sequencing or RNA-sequence-based next-generation sequencing. Pyrosequencing, Polony sequencing (Polymerase colony sequencing), massively parallel signature sequencing, and oligonucleotide ligation-based sequencing are some recent and advanced approaches in HTS (Rajesh and Jaya 2017).

10.7.1 DNA-Seq-Based Next-Generation Sequencing

Isolation of DNA and its fragmentation, library construction, sequencing, bioinformatics analysis, mutation annotation as well as data analysis are the foremost requirement in performing DNA-seq-based NGS to identify a particular targeted gene to detect phytopathogens (Qin 2019).

10.7.2 RNA-Seq-Based Next-Generation Sequencing

RNA-seq is an innovative and advanced technology that explores the dynamic nature and expression level of mRNA transcripts (transcriptome) with high resolution. RNA-seq is based on converting a large RNA population into cDNA library through reverse transcription. Then cDNA is fragmented, followed by ligation of the adaptor at either one or both ends for sequencing purposes. Sequencing can be done with amplification and without amplification of cDNA. Sequencing can be performed either from a single end, called single-end sequencing or from both ends, called pair-end sequencing. Reads are small segments of DNA or RNA, usually 30–400 bp in size, depending on which sequencing approach is used. After that, a library is constructed to determine the targeted RNA transcript. For library construction, fragmentation of cDNA/ RNA is necessary to perform NGS further.

Then, the targeted mRNA transcript is primed with either oligo primers or random primers for reverse transcription reaction. Oligo primers are preferred over random primers because of the addition of poly-adenylated mRNA that gives precise information (Mortazavi et al. 2008). After sequencing, subsequent reads are aligned to the reference genome sequence or amassed de novo to generate a genome-scale map of mRNA transcripts which comprises information on gene structure as well as expression level. Trinity and Rnnotator are software that align the RNA-seq data by assembling the resultant reads without a reference genome. This approach helps to find the novel transcripts from various sources (Grabherr et al. 2011).

Most generally used NGS platforms for RNA-seq are SOLiD, Illumina's HiSeq, Ion Torrent, and Illumina MiSeq, which has set a standard for NGS (Wang and Peng 2014; Kukurba and Montgomery 2015). RNA-seq tool can be used to identify and detect phytopathogens, particularly fungal pathogens causing disease. In previous studies, a whole-genome NGA approach was established through Illumina MiSeq platform to diagnose a novel pathogen, *Calonectria pseudonaviculata*, causing Sarcococca blight in ornamental plants (Malapi-Wight et al. 2016). *Magnaporthe oryzae* that results in rice blast disease (Soanes et al. 2012), *Verticillium dahliae* that causes vascular wilt disease in tomato (de Jonge et al. 2012), *Puccinia striiformis* that causes wheat yellow rust disease (Hubbard et al. 2015), *Pseudoperonospora cubensis* and *P. humuli* that cause cucurbit downy mildew disease are detected through DNA/ RNA seq-based NGS approach (Withers et al. 2016). In another study, a hierarchical and hybrid *de novo* association analysis was performed by combining Pacific Biosciences (Pac Bio) and Illumina platforms to diagnose *Monilinia fructicola*, causing brown rot disease (De Miccolis Angelini et al. 2019). Using this hybrid approach, another rust fungal pathogen (*Hemileia vastatrix*) genome was sequenced using Illumina HiSeq and PacBio RII sequencing platforms (Porto et al. 2019). This reference genome sequence could be used for the identification of other species. If no prior knowledge is available for a novel pathogen, the whole genome can be sequenced without primers and PCR amplification (Hadidi et al. 2016; Malapi-Wight et al. 2016). With the advancement in single nucleotide-based sequencing, third-generation sequencing is preferred over second-generation

sequencing technology (Schadt et al. 2010). Time consumption, low RNA yield, RNA stability, integrity, impurities with chemicals, and DNA are some major factors in HTS that limit its application in phytopathogens detection (Cortés-Maldonado et al. 2020).

10.8 DNA Barcoding

DNA barcoding is a multipurpose molecular tool for diagnosing phytopathogens using a particular segment of DNA. These particular sequences (~ 600 bp) are barcode regions unanimously present in target lineages (Gao and Zhang 2013). It shows considerable sequence variation to distinguish various pathogens (Krishnamurthy and Francis 2012). It has been proven quite efficient for identifying symptomless plants and pathogens in their latent period. DNA barcoding has been productively used to identify fungi, bacteria, and viruses. DNA barcoding based on PCR, LAMP, RCA, and other molecular techniques has revolutionized the pathogen detection approach (Choudhary et al. 2021). Its efficacy depends on higher genetic variations present within species than between species. Fungal morphological identification is not effective, so DNA barcoding gives promising results for diagnosing fungal pathogens (Roe et al. 2010). Internal transcribed spacer (ITS) is an ideal barcode for identifying fungal phytopathogens (Schoch et al. 2012) such as *Sclerotinia trifolium* causing stem crown rot disease in *Trifolium alexandrinum* (Faraz et al. 2021). Hsieh *et al*. (2020) used an amalgamation of ITS, histone H3, and β-tubulin barcode regions to diagnose *Cercospora*, *Fusarium*, and *Colletotrichum* species in *Platostoma palustre* plant.

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Microarray Technology for Detection of Plant Diseases

11

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Abstract

DNA microarrays are a powerful technology that has great implications in plant sciences, biomedical sciences, veterinary, and pharmacological research. It can deal with large numbers of samples for the detection of microbes, clinical diagnosis, gene expression analysis, host–pathogen interactions, food safety testing, and environmental monitoring. Detecting and quantifying the microbial populations in microbial ecology are difficult due to practical difficulties. Conventional procedures such as dilution plating and biochemical assays are ineffective in identifying bacteria and yeasts at the species level and in identifying multiple species simultaneously. These techniques are also imprecise, time-consuming, and unable to identify unculturable species. This chapter focuses on

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the review of applications of DNA microarrays for identifying the plant pathogens, fungi, viruses, bacteria, mycoplasma-like organisms, and nematodes. In this chapter, we discuss the gene expression profiling of pathogens and their host plants, new virulent and resistance genes discovery, regulation of gene networks, and physiological pathways under biotic stresses. The supremacy of microarray technology has unraveled the unprecedented knowledge about plant pathogens and their interactions with the host plants, creating new research avenues in the field of molecular plant pathology.

Keywords

Genomics · cDNA · Microarray chip · Probes · Pathogen identification

11.1 Introduction

Plant diseases are one of the most significant constraints to global agricultural production. Detecting plant pathogens responsible for causing diseases early and keeping a close eye on plant health are critical to limiting disease development and dissemination and applying appropriate management measures successfully. While plant pathogens can be identified morphologically, use of serological and nucleic acid-based techniques can increase accuracy and specificity including the pathogens that lack visible morphological structures. Since the development of protein and nucleic acid-based diagnostics, pathogen identification has been revolutionized, and plant disease diagnosis has substantially been improved. DNA microarrays are a valuable tool for studying gene expression, which enables researchers to monitor great amounts of RNA produced in thousands of distinct cell types. This is accomplished using a reverse transcription process to produce complementary DNA copies of the RNA transcripts found in a tissue sample. These complementary DNA copies can then be spotted onto slides using one or more color-coding systems that correspond with patterns of gene expression algorithms.

Biologists have shown great interest in the potential applications of DNA microarrays since DNA microarrays were first introduced in 1995. Microarray technology is an important tool for genetic research because it can examine the expression of hundreds or thousands of genes simultaneously. Microbial research, drug discovery, clinical diagnostics, and genetics are examples of the fields where this technology has been being widely applied. DNA microarrays are expected to be used for the detection and identification of a wider range of plant diseases because of the explosion of knowledge gained by sequencing plant pathogens (Mitra 2021).

It has been 51 years since Watson and Crick first discovered the phenomenon of "DNA complementarity," which allows DNA molecules to be applied to solid surfaces. Gillespie and Spiegelman's 1965 method allows DNA to be immobilized on a membrane and to bind to cRNA or DNA through particular hybridization. Southern and Mitchell's (1971) technique is an obvious extension of the DNA array technique, in which DNA is put to an untreated cellulose surface and then hybridized

(Southern and Mitchell 1971; Southern 1975) while southern blotting is coined as a result of the latter method. According to Kafatos et al. (1979), dot blot hybridization can be utilized in various ways to determine the homology and concentration of nucleic acid sequences. These processes explain how to deploy the probes that have been identified by a signal, whether they are radioactive or fluorescent in nature. Massive parallel observation of hybridization processes is possible due to applying substantial quantities of DNA oligonucleotides to surfaces in two-dimensional arrays. Using microarrays for quantitative gene expression measurements was reported initially in 1995 by Stanford University researchers (Schena et al. 1995). Microarrays and other techniques like quantitative PCR and serial gene expression studies were presented as a high-throughput, parallel approach for measuring gene expression levels of multiple genes. More articles utilizing microarray technology have been published since 1995, with 600 articles in 2001 (Afshari 2002).

11.2 Principle and Types of Microarrays

DNA microarrays were developed on the principal that complementary base sequences can be hybridized to form base pairs. For a specific complementary DNA probe to be found on the array, the target's DNA must bind to a specific complementary DNA probe. Each of these probes contains hundreds of cDNA molecules or oligonucleotides specific to a particular gene, DNA sequence, or RNA sequence. Nucleic acid samples can be identified using base-pairing principles based on the DNA and RNA sequences. The DNA probe is deposited on a solid platform, and a few nanoliters are used to create each array. Using a robot for printing guarantees that the spotting can be done consistently (Missoum 2018). An array is referred to as a macroarray or microarray, depending on the number of sample places. Microarrays typically have sample spots of at least 300 nm in diameter. There are generally hundreds or thousands of dots on a microarray sample location that is less than 200 nm in diameter.

Microarrays with the density of 100 spots per cm^2 or less are sometimes referred to as "low-density." There are many commercial suppliers of the low-density arrays, as well as many labs producing their own microarrays. For the low-density arrays, glass is often used since nucleic acids cannot adhere to it without treatment. Ionic interactions are used to affix molecules to poly-L-lysine for this purpose. If the glass substrate has been pretreated with saline, then the probe DNA will be bound covalently to the saline and will not be removed during the hybridization and washing stages. A nylon membrane can cross-link DNA probes covalently to glass slides used to detect radioactively labeled nucleic acids. Plasmids, complementary DNA, PCR products, or synthesized oligonucleotides with amino or thiol groups on their 5′ ends can all be used to apply DNA to the array's surface. Plasmids can range from 500 to 5000 bases (Hadidi et al. 2004).

A microarray is considered "high-density" if it has a density of 1000–10,000 spots per cm^2 or more. It is common to refer to the high-density arrays of DNA as "DNA chips." Chemically modified glass or silicon is commonly utilized as the

substrate for immobilizing high-density DNA arrays. When thousands of nucleotides can be applied to a surface in an ordered array, it becomes possible to interrogate thousands of different sequences simultaneously, and this is the reason why DNA microarray technology shows its superiority.

11.2.1 Types of Microarray

The simultaneous detection of the expression of numerous genes or proteins is made possible by the use of microarrays in various lab settings. In a short time, microarrays can examine a large volume of biological materials despite their small size and high complexity. Multiplexed parallel processing methods can boost throughput and generate more data in a single microarray experiment. All kinds of microarrays have identical methodologies and analysis techniques, but the goals and uses of each microarray type differ significantly.

11.2.2 DNA Microarrays

The DNA microarrays are the most commonly used type of microarray in scientific research. It is possible to diagnose diseases and inherited anomalies using DNA microarrays that are particular to the genome. An analyst uses a small glass plate to print hundreds of synthetically generated short single-strand DNA sequences to create these DNA microarrays. Supplementing the synthetic sequence with genetic material from a plant is done in the same manner. Using a synthetic DNA fragment, the analyst can detect and identify specific mutations in the plant's DNA. To better understand the role of gene mutations in diagnosing various diseases, practitioners typically employ DNA microarrays to research and identify multiple gene mutations (Marzancola et al. 2016).

11.2.3 Protein Microarrays

Although DNA microarrays can be used to detect gene alterations, they cannot be used to measure gene expression in individual cells accurately. To address the shortcomings of DNA microarrays, protein microarrays were developed. As a result, protein microarrays concentrate on the protein–protein interactions rather than the mRNA–mRNA interactions. Many of the same analysis techniques used in DNA microarrays are used in protein microarrays to examine the interactions among proteins and establish their role at both the micro and macro levels (Popescu et al. 2009).

11.2.4 Peptide Microarrays

Because of their parallelism, peptide and protein microarrays' techniques and uses are likely to be highly comparable. Similar to protein microarrays, peptide microarrays are used to study the protein–protein interactions. In contrast, peptide microarrays are also ideal for tracking antibody epitopes and revealing protein-binding residues. These microarrays are used extensively in medicine, biology, and pharmacology and can contribute to the improvement of more accurate diagnostic tools and vaccinations (Lodha et al. 2013).

11.3 DNA Microarray Experiment Design and Implementation

A DNA microarray experiment necessitates a wide range of steps in the planning and execution process. These are the steps that you'll need to follow:

11.3.1 Sequence-Specific DNA Probes

Probes can be oligonucleotides of any length, cDNAs, chromosomes, or even whole organisms. Standard techniques, including restriction enzyme digestion, cloning, and polymerase chain reaction (PCR), can be used to make the probes. Gene expression libraries can either be purchased or synthesized, depending on the needs of the experiment. There are thousands of these different cDNAs or oligonucleotides in a single probe, each targeting a different gene or piece of DNA/RNA. The baker's yeast *Saccharomyces cerevisiae* was used to create arrays comprising oligonucleotides representing the known genes. There were more than 65,000 DNA synthesizing features in the arrays. An oligonucleotide of 25-mers, each containing more than 10^7 copies, is used to synthesize each 50–50 μm feature. Oligonucleotides for each yeast's 6321 known genes have an average of 40 synthesis traits (Lipshutz et al. 1999; Mirmajlessi et al. 2015). Oligonucleotides specific to the 30,000 human genes have been created using an approach similar to this (http://www.mwbiotech.com/html). However, it is possible to detect the role of novel unexplored and un-sequenced genes, including those in viral genomes, using cDNAs.

11.3.2 Expansion and Application of DNA to Arrays

Contact-dip deposition printing, micro-contact printing, electrocapture, and microfluidics networks can all be utilized to immobilize DNA on the array's surface. Immobilization and in situ synthesis are two possible applications for piezoelectric printing and micro wet printing. The photolithographic method is the only way to perform in situ synthesis. A few nanoliters of highly purified DNA are deposited onto a solid support to construct a DNA array. Robotic printing uses a series of

identical patterns to create a finished object. As opposed to those used for microarrays, the supports for macroarrays can have up to 2–3 cm^2 and tens of thousands of genes per cm^2 (Bertucci et al. 2001). Oligo-chips, often called gene chips, were developed by Affymetrix as a technology (www.affymetrix.com). One of their first ideas was to chemically synthesize single-stranded DNA segments of 20–25 mers to generate polynucleotides directly on a rigid framework directly. Photolithography and solid-phase DNA production are required to make these arrays. It is necessary to have a light source for these two operations. Various bases are attached and joined to a glass substrate covered in synthetic linkers. Anywhere on the glass substrate can be used to generate DNA probes by using this method. By utilizing this technology, it is possible, for example, to build arrays containing hundreds or thousands of polynucleotides in a single cm^2 (and as much as one million in experimental prototypes) (Lipshutz et al. 1999).

11.3.3 Target Preparation

The first single-stranded cDNA is reverse transcribed using either purified poly (A+) RNA or the RNA in its entirety. The target dCTP tagged with Cy3 or Cy5 fluorescence is integrated into the cDNA when the cDNA is reverse transcribed on a glass support. An alternative to reverse transcription is to employ "post tagged" cDNA, which binds to the nucleotides that have been altered by Cy3- or Cy5-NHS ester reactive forms. Microarray hybridization with these cDNAs yields homogeneous and high signal intensities. Autoradiography or chemiluminescence can be used to detect the hybridization of cDNA to nylon membranes containing radiolabeled nucleotides such as a32 P-dCTP (Bertucci et al. 2001; Heller 2002). To label cDNA after hybridization, biotin-16-dUTP and digoxigenin-11-dUTP can be utilized; hybrids are detected by colorimetry using streptavidin-coated β-galactosidase or an alkaline-phosphatase conjugated anti-digoxigenin antibody (Chen et al. 1998). To prevent interference with the detection of the radioactive and fluorescent signals, RNA purity is required at this experiment stage.

11.3.4 Hybridization

The labeled target solution is made using a hot denaturation of the probe DNA. Nylon membranes require between 300 ng and 5 g of original total RNA for hybridization, while glass surfaces require up to 20 g of original total RNA. A single dye or radioactive labeling hybridization is sufficient if the array is hybridized to a single cDNA type. One can use either Cy3-dCTP or Cy5-dCTP to mark the control and experimental cDNA samples, depending on the objective of the array. Both cDNA and dye must be used in equal proportions to acquire accurate results. Hybridization occurs in a 65 °C humid environment over a long period. Finally, the slide or chip is sanitized to eliminate unbound nucleotides so that the detection stage can proceed (Singh and Kumar 2013).

11.3.5 Detection of Images

Scanners and direct imaging systems are both used for detection. A laser excitation source and a detector measure the light emitted by this excitation source in each place. This allows the identification of fluorescently labeled DNA. An image can be captured and preserved using direct imaging, in which a single excitation source illuminates the entire array simultaneously. It can be utilized with radioactive hybridization or colorimetry (Zhao et al. 2014). The resolution and sensitivity of direct imaging are surpassed by scanning. The detection findings build graphic computer files (such as TIFFs).

11.3.6 Data Analysis

The DNA array data must be presented in the most informative manner possible. Complex bioinformatics methods are needed for this, and it is necessary to use specialized software to determine the light intensity at each detected wavelength when dyes are used. The Cy3/Cy5 ratio is assessed by scanning the slide for fluorescence of Cy3 and Cy5 cDNAs that have been hybridized. When cDNA from different populations hybridizes together, the result is an artificial color overlay that looks red or green depending on how much of one group hybridizes with the other. Although each cDNA is represented by "red" and "green," the relative abundance for each gene is represented by the ratio of "red" to "green" (experimental vs. control). The expression levels of experimental and control samples are compared using these metrics. When looking for image analysis software, one can find a non-exhaustive list here: ArraySoft Image Analysis Software (http://ihome,cuhk.edu;b400559/ArraySoftImage.html). This includes a two-dimensional grid with changing hues of red, green, and yellow dots that correspond to the points in the array. Instead, a scatter plot can be generated to demonstrate the differences in gene expression levels. To make sense of large amounts of data, it is necessary to use technologies like GeneSpring and Spotfire (Amaratunga et al. 2014).

11.4 Application of Microarrays

Microarray technology is extremely useful in the field of genetic study. Research in biological, pharmaceutical, genomic, and diagnostic fields, as well as in diagnostics and food quality and safety, are all benefiting from its use. Microarrays are most commonly used for the following purposes:

11.4.1 Gene Expression Analysis

Multigene expression can be studied using microarrays, which have proven to be a highly effective tool for this task. For studying the simultaneous expression of up to 15,000 ESTs, it is preferable to use cDNA-based microarrays, especially if the ESTs have not yet been sequenced. EST sequencing is only done when the ESTs show distinctive gene expression patterns. Concerning specificity and sensitivity, oligonucleotide-based microarrays are superior to cDNA-based microarrays. *Drosophila*, *Saccharomyces cerevisiae*, and *Arabidopsis* have been extensively studied utilizing DNA microarrays for gene expression profiling (White et al. 1999; Beisson et al. 2003; Marzancola et al. 2016).

11.4.2 Applications in Agriculture

Plant genetics and gene functions are currently being studied by using microarray technology. Genetically engineered food can be tested by using the first commercially available DNA chip (Bawa and Anilakumar 2013). The chip can detect genetically modified organisms (GMOs) in raw materials, processed foods, and animal feed. As a consequence, it can identify viral DNA (CaMV) in addition to particular gene segments, such as the Nos-terminator gene (Bt, EPSPS).

11.4.3 Food Quality

Public health, economics, religion, and the law are all affected when tissues from animals of unknown species are found in food. It is now possible to distinguish between 33 different species of animals using an array of DNA chips, which comprises 80,000 oligonucleotide probes and is available from Affymetrix http://www.affymetrix.com/orporate/media/genechip_essentials/foodexpert/FoodExpert_ID_Array.affx).

11.4.4 Food-Borne Pathogens

Microarray testing revealed several different bacterial pathogens that can be transmitted through food. In addition to *E. coli* O157:H7 and STEC, *Campylobacter, Salmonella typhimurium* DT 104, and *Listeria monocytogenes* (http://www.gov.on.ca/OMAFRA/english/research/specialresearch/2001/sr9087.htm) are just a handful of the pathogens that have been detected so far.

11.4.5 Detection of Microorganisms

As many as a thousand microscopic organisms can be quickly and simultaneously identified using DNA microarray technology. The microarray can detect the microorganism infecting plants, humans, animals, and microbial populations in environmental samples.

11.5 Applications of Microarray Technology

11.5.1 Microarray-Aided Microbial Diagnostics

Plant viruses, viroids, and phytoplasmas must be detected and identified for epidemiological and genetic investigations, pathogen comparisons, quarantine and certification, and as a follow-up to pathogen therapy (Hadidi et al. 2004; Ragozzino et al. 2004; Martinelli et al. 2015). Methods for detecting each infection listed above have been developed over the past few decades. There are four main types of these strategies. Herbaceous and woody indicators are common in conventional biological indices. To accurately identify pathogens, this approach has several shortcomings and is the most commonly used to detect one pathogen per experiment. An antibody and an antigen must interact to identify one pathogen at a time in a gel diffusion, an ELISA, or an immunoblotting assay. It is impossible to utilize these procedures on viroids since they are infectious naked RNAs without coat proteins; hence, they are unsuitable (Pallas et al. 2018). One pathogen can be identified with each experiment using cDNA and nucleic acid hybridization probes, and PCR and RT-PCR are also included in this group.

Most applications benefit significantly from the PCR's high level of selectivity while also delivering good sensitivity. The limitation is that only one organism can be addressed per response tube even with enhanced multiplexing. It's been more than a decade since researchers began working on various virus- and other pathogen-detection initiatives in plants (Nikitin et al. 2018; Pathak et al. 2020). Plant viruses and other plant pathogens can be detected by using DNA microarray technology. Additionally, studies have been initiated to find "bacteria" and *Fusarium* spp., many of which produces mycotoxin (Nicolaisen 2002), and *Aspergillus* spp., some of which produces aflatoxin (Scherm et al. 2002), and nematodes (Petralia et al. 2022), as well as other plant pathogens. Commercial DNA microarrays were produced by Agilent Technologies and North Carolina State University using DNA probes from the rice blast fungus *Pyricularia oryzae* and the rice plant itself. For the first time, researchers may analyze the interplay of diseases on plants by using microarrays with two genomes.

Plant viruses, viroids, phytoplasmas, and other diseases infect commercially important horticultural and field crops and ornamental plants. These pathogens are divided into 16 families and 65 genera, respectively. Current methods for screening a wide variety of viruses, viroids, and phytoplasmas can only detect a small number of potential pathogens, and one pathogen is usually the focus of each experiment, as

previously mentioned. International efforts are underway to design and manufacture a DNA microarray chip based on these pathogen genomes' available nucleotide sequence data. This method may simultaneously identify viruses, viroids, virusoids, and phytoplasmas. Hybridization patterns can be used to identify diseases that are closely related. It is feasible to select microarray components derived from pathogen families, genera, or groupings to detect pathogens not expressly represented in the DNA microarray. Pathogens, particularly those engaged in plant diseases with an unknown etiology, can be discovered using this strategy. Because of versatility and capability of detecting a wide range of infections at once, DNA microarrays are an excellent tool for determining which pathogens have been found (Amiri et al. 2018). Plant virology research, certification, and quarantine programs can benefit from this technique. This technique may reduce sample preparation, the time required for assay and data analysis, and costs.

Most applications of microarrays have been far focused on detecting the diversity of soil pathogen populations. Black root rot disease-suppressing or conducive bacterial communities in soil (*Thielaviopsis basicola*) can be used as an example for illustration. Kyselkova et al. (2009a) created an array of microarrays with 1033 probes specifically tailored to target 19 different bacterial phyla, such as Proteobacteria and Firmicutes. Researchers could separate soils with suppressive or favorable black root rot using microarray data. No follow-up studies have been published, which uses the microarray developed by Lievens et al. (2005) to measure the levels of the vascular wilt pathogens *Verticillium albo-atrum* and *V. dahliae* in the soil. There has been much discussion regarding the challenges posed by base-pair mismatches and the fact that the signal intensity is dependent on the probe. Both of these problems can be fixed by using probes that can only ligate to targets that are an exact match for them (Kyselkova et al. 2009b). This approach does not allow for the quantification of individual bacteria inside a sample but allows comparisons between samples.

11.5.2 Plant–Pathogen Interactions

Genomic technologies enable the study of complete developmental or metabolic processes, gene networks, chromosomal locations of key genes, and their evolutionary history. For example, transcript profiling has provided insights into plant–pathogen interactions, such as the mechanisms of host/non-host resistance and biotrophy vs. necrotrophy, as well as the pathogenicity of vascular and non-vascular infections. With microarray technology, it is possible to find commonalities and differences in host–pathogen interactions on a wide scale (Wise et al. 2007).

11.6 Cellular Perspective in Biological Stress Pathways

Methyl jasmonate (MeJA), as a plant defense gene regulator, has been shown to provide long-lasting and highly targeted effects. Compared to other signaling molecules, MeJA has been examined more extensively. *Arabidopsis thaliana* and other systems have been used to study the molecular genetic pathways in which MeJA is engaged, including microarray analysis, knockout lines, and functional gene expression investigations (Pickett et al. 2007).

Transgenic *Arabidopsis* plants overexpressing the jasmonate carboxyl methyltransferase gene were subjected to gene expression analysis using GeneChip arrays (*AtJMT*). Genes associated with defense and oxidative stress tolerance were among 80 upregulated genes, whereas genes involved in photosynthesis and cold/drought stress responses were among 88 genes with reduced expression. According to the findings of this study, MeJA is a key regulator of jasmonate-responsive gene expression in plants (Jung et al. 2007a).

Another study found that adventitious roots of *Bupleurum kaoi* are regulated by interactions between signaling pathways. Stress-resistant roots of *B. kaoi* were shown to have a higher concentration of MeJA-activated genes involved in the synthesis of saikosaponin, amino acids, phenylpropanoid, and jasmonate biosynthesis. An *Arabidopsis thaliana* jasmonate sensitive gene screen found that when applied externally to the rosette leaves of 5-week-old plants, 100 mM MeJA significantly affected the expression levels of 137 genes (Jung et al. 2007b). Cell wall alterations, defense, oxidative stress responses, and jasmonate production were all elevated in that study, whereas there was a downregulation of genes involved in chlorophyll production and photosynthesis. Abiotic stress responses, jasmonate, and abscisic acid signaling pathways were antagonistic.

The transcriptome of sugarcane was studied using cDNA microarrays containing 1545 sugarcane genes. One or more treatments showed differential expression in 179 investigated genes (Rocha et al. 2007). The results of ABA and MeJA tests also revealed that genes associated in signal transduction, transcription factors (TFs), hormone production, new genes, and genes relating to undiscovered proteins made up most of the arrayed elements.

Defense mechanisms against biotic stresses were identified by measuring the levels of jasmonic acid (JA) and salicylic acid (SA) after herbivore attacks on *Nicotiana attenuate* (Rayapuram and Baldwin 2007). As a result of SA treatment, the transcript level of *NaNPR1* increased, making plants more vulnerable to herbivore and disease attacks. An analysis of microarray data found increased regulation of genes involved in SA production in plants silenced by NPR-1, but decreased regulation of several JA-induced genes. It was found that *NPR1* negatively regulated SA production during herbivore attack, triggering JA-mediated defenses; as a result, the SA production of NPR1 silenced plants was enhanced, whereas the plants' protective mechanisms against herbivory were weakened.

Auxin, a vital plant hormone that regulates plant growth and development, may affect a plant's vulnerability to disease. According to the plant's genomic annotation, *A. thaliana* GH3 auxin-responsive gene family, GH3.5, has in vitro adenylation

activity for both IAA and SA (Zhang et al. 2007). In response to pathogen infection, GH3.5 functions as both a stimulator and an inhibitor of SA by increasing the expression of SA-responsive genes and defense components and decreasing the expression of auxin repressor genes.

11.6.1 Symbiosis and Fungi-Induced Infections

Pathogen invasion triggers a series of signaling pathways that induce systemic resistance in a distant tissue. *Arabidopsis brassicicola*-induced systemic defenses in *Arabidopsis* plants resulted in the upregulation of 25 genes and the downregulation of 10 genes in the distal plant tissue (Schenk et al. 2003). Distal tissue alterations in genes with putative functions in cellular housekeeping reveal that plants modify these processes to allow for a coordinated pathogen response. Signal transduction and cell wall formation/modification genes were transcriptionally upregulated. The study found that distal tissues of pathogen-challenged plants are well prepared for future attacks.

Fusarium oxysporum f. sp. *vasinfectum* (Fov)-infected cotton hypocotyl tissues express more defense-related genes than infected root tissues. More genes are inhibited than activated in infected roots, especially early on. An early study showed that pathogens suppress drought-responsive proteins in roots and hypocotyls, and this response leads to vascular wilt disorders. The gene expression study revealed that the phytohormones ET and auxin have a role in the progression of the disease (Dowd et al. 2004).

Microarray and qRT-PCR were used to identify genes expressed in cotton root and hypocotyl tissues during pathogenic Fov infection. There were 218 fungus clones representing 174 genes for Fov expressed in plants. Few Fov genes were known or linked to fungal growth and energy production. Eleven fungal genes were selectively expressed in plant tissue, including a suspected oxidoreductase gene with homology to *Agrobacterium tumefaciens*' AtsC (Mcfadden et al. 2006).

Resistance to SA, MeJA, ACC, *Ascochyta rabiei* was investigated in chickpea. It was shown that the three *Cicer arietinum* genotypes had different levels of Ascochyta blight resistance (Coram and Pang 2007). According to the study, the *A. rabiei*-resistant genotype had higher levels of defense-related gene activation, which identified differential expression in 425 transcripts. Chickpea defense mechanisms may be better understood, and disease-resistant cultivars may be developed due to these findings on the molecular control of cellular activities in chickpeas.

More than a quarter of *Arabidopsis* genes were altered by these 7000 ESTs, which were tested in various mutant backgrounds and infected with the oomycete pathogen *Peronospora parasitica* or treated with the chemical benzothiadiazole. *Arabidopsis* mutants Cim6, Cim7, and Cim11 all showed the same patterns of gene expression as BTH-expressing wild-type plants. There was a 2.5-fold difference in the expression patterns of 413 ESTs.

Maize gene chips have been used to study the Les9 maize mutant's differential gene expression, which includes the generation of defense-related proteins and better

resistance to *Bipolaris maydis* in maize (less than 9%) (Nadimpalli et al. 2000). Infection with *Cochliobolus carbonum* activated or repressed 117 genes on an Affymetrix Gene Chip encoding 1500 maize ESTs (Baldwin et al. 1999). One-thirds of the genes on this array were defense-related, based on their homology to known or suspected defense-related genes. A total of 69 genes, several previously linked to plant defense, had mRNA abundance changes of at least two-fold. Individual gene names remained a mystery except for Zm-hir3, which is thought to be involved in cell death via ion channel control.

11.6.2 Rust Diseases

Microarray transcriptome profiling was performed for 3-week-old soybean plants infected with the Asian soybean rust pathogen (Panthee et al. 2007). The transcriptome profiling resulted in the defense and stress-related differential expression of 112 genes, including upregulation of 46 genes while six were downregulated. The plant's inadequate and non-targeted response to the virus may be accountable for its inability to develop resistance to soybean rust.

Many wheat diseases, including leaf rust, yellow rust, and others, are protected by the *Lr34/Yr18* gene in wheat. In the absence of *Lr34/Yr18* precise role, a microarray-based study was conducted on wheat near-isogenic lines for *Lr34/Yr18*, which were inoculated and mock-inoculated on the flag leaf, respectively (Lagudah et al. 2009). The mock inoculation of bacteria caused the upregulation of ABA deficiency-related genes in the leaf tips of the resistant lines, while no upregulation of PR proteins was observed in the mock-inoculated resistant lines. The upregulation of PR genes in the rust inoculated lines reflected the putative role of *Lr34/Yr18* in imparting resistance.

11.6.3 Plant Virus–Host Interactions

Plant viruses have varied nucleotide sequences and induce diverse plant diseases. Microarray technology allows for specialized virus testing. Virus infection triggers many signaling pathways in host cells. A plant's resistance and vulnerability to viruses are reflected in the cellular response. Viral protein accumulation causes non-specific changes in gene expression although specific virus–host protein interactions also have an effect. Stress and defense-related genes are among those whose expression changes in response to viruses, while the viral RNA silencing suppressors modulate the gene regulation and hormone signaling leading to abnormal development of the host (Rahman et al. 2021).

Microarrays were used to measure *Nicotiana benthamiana's* gene expression changes after infection by enveloped viruses. In the case of *Impatiens necrotic spot virus* (INSV), 275, 2646, and 4165 genes were differentially expressed 2, 4, and 5 days post-inoculation, respectively, while 35, 665, and 1458 genes had varying expression after 5, 11, and 14 days when inoculated with *Sonchus yellow net virus* (Senthil et al. 2005).

Cucumber mosaic virus (CMV), tomato mottle virus (TMoV), potato virus X (PVX), turnip vein-clearing virus (TuVCV), and odontoglossum ringspot virus (ORSV) were all used to infect *Arabidopsis* plants. The microarray analyses showed the coordinated differential expression of genes in case of multiple virus infections. According to gene promoter investigations, the RNA viruses that infected susceptible host plants responded similarly through unknown signaling mechanisms (Whitham et al. 2006). The susceptible potato lines infected with tuber necrotic ringspot disease, caused by *Potato virus Y*, were subjected cDNA microarrays analyses. The analyses showed the expression of different genes, but notably Igor gene expression was most prominent at 14 dpi (Pompe-Novak et al. 2005).

Viruses in a variety of ways alter plants' molecular and morpho-physiological characteristics. Tomato ringspot nepovirus, Prunus necrotic ringspot ilarvirus, and plum pox potyvirus exhibit various symptoms. The comparative expression profiling was conducted to discover if there were any gene expression changes in *N. benthamiana* infected with three fruit tree viruses. Tomato ringspot virus (ToRSV), prunus necrotic ringspot virus (PNRSV), and plum pox virus (PPV) infection had been able to regulate expression of 1082, 744, and 89 genes. Moreover, the PPV- and ToRSV-infected leaves inhibited plastid-related genes. PPV enhanced cytosolic ribosomal gene expression while ToRSV reduced plastidic ribosomal gene expression (Dardick 2007).

11.6.4 Maladies caused by Bacteria

Arabidopsis was one of the first plants studied for bacterial effects on gene expression. The cDNA microarray analysis showed an increase in defense-related transcripts in *Pseudomonas thivervalensis*-infected *Arabidopsis* shoots (Calvo et al. 2019).

Basal resistance to the bacterial infection in *Arabidopsis,* microarray expression profiling was done to ascertain the role of WRKY53 transcription factor and HSPRO2-like nematode resistance protein against *P. syringae* pv. *tomato*. The two tomato mutant lines, lacking *At4g23810* and *At2g40000* genes, responsible for coding WRKY53 transcription factor and HSPRO2-like nematode resistance protein, were proved more vulnerable to infection by *P. syringae,* demonstrating the positive role of these genes in incorporating resistance. These two genes interfered with the SA, JA, and ET signaling (Murray et al. 2007).

The genomic expression analyses of 7981 tomato and pepper varieties resistant to *Xanthomonas campestris* pv. *vesicatoria* (Xcv T3 bacteria) causing spot disease were carried out in Hawaii. The differentially expressed genes were classified, on a functional basis, into 20 functional groups, which also included stress and defensive responsive genes, apart from protein synthesis and signaling genes. The *AvrXv3* effector protein was found to directly regulate 77% of the XRE (Xanthomonas-regulated) genes studied in a comprehensive analysis. Following an incompatible interaction with an avirulent strain of *P. syringae* pv. *tomato*, 64% of the XRE genes were activated in tomato (Gibly et al. 2004).

Hypersensitive response (HR) was determined using cDNA from infected and non-inoculated cotton line Im216 leaves. PCR was used to amplify unique sequences and genes, which were then placed on glass slides. After 8–60 h post-inoculation, these microarrays were used to examine Im216 transcripts. Over 98% of the genes showed an increase in expression during one or more sample periods. The upregulated genes, a total of 63%, were involved in protein synthesis, disease or defense-related PR proteins, secondary metabolism, or retrotransposon-like protein (Da Silva and Yucel 2008).

11.6.5 Nematode-Induced Changes in Host Plants

Genome-wide gene expression analyses of the soybean cyst nematode (SCN) and its host plant were conducted simultaneously. Of the 35,611 soybean genes on GeneChip arrays, many were up/downregulated in infected root tissues. Genes that encode enzymes involved in primary metabolism, lignin and flavonoid production and the stress and defense response system, alterations to the cell wall, cellular signal transduction, and transcriptional control were some of the genes discovered. Among 7431 SCN transcripts, more than 1850 were expressed differentially in different phases of worm parasitism and development (Ithal et al. 2007).

The root-knot nematode, a plant parasite known as *Meloidogyne javanica*, creates and maintains a long-term root inhibiting feeding site. Through the mutually beneficial interactions of Meloidogyne species, roots are transformed into multinucleate feeding cells for worms (also known as "giant cells"). The root-knot nematode-infected tomato was subjected to gene expression analysis through a tomato-spotted microarray chip (Bar-Or et al. 2005). Researchers found that the steady-state transcript levels of various functional categories, such as PR genes, hormone-related transcripts, and development-relevant transcription factors (TFs), had been altered. Research on the plant response to root-knot nematodes was conducted using large cell-enriched root tissues from *Arabidopsis* (Jammes et al. 2005). A substantial difference in gene expression was found in 3373 of the 22,089 genes examined. The down regulation of specific genes may also be necessary for the appropriate formation of galls. Jammes et al. (2005) discovered that nematode feeding sites were formed due to the suppression of plant defenses.

11.7 Cataloguing Host Responses

Gene expression analysis in plants is only getting started with microarray technology (Heller 2002; Behzadi and Ranjbar 2019). Initial microarray-based experiments have paved the way for further functional studies which could unlock the critical mechanisms underlying plant defenses. Expressed sequence tags (ESTs) of 2375 *Arabidopsis* genes were recently analyzed to see if there were any transcriptional changes following inoculation with the incompatible fungal pathogen *Alternaria brassicicola* or the application of low molecular weight signals like methyl

jasmonate or ethylene. Seven hundred five mRNAs varied at least 2.5-fold in response to one or more treatments in this study. One hundred six genes were discovered with previously unknown functions, despite the majority of these genes being identified based on their similarity to previously known sequences (Schenk et al. 2000). It has been found that treatment with SA and marijuana (MJ) enhanced transcript levels in 192 genes and suppressed transcript levels in another 221 genes. There are genes involved in antimicrobial defense, cell wall remodeling, phytoalexin synthesis, and signal transduction. These stimuli may have induced secondary effects because just a one-time point was used in this investigation. *A. brassicicola* (incompatible pathogen) gene expression patterns were compared to those of tissue that had not been injected. The findings showed that 72 h after inoculation, 168 gene transcripts were specifically induced in infected leaves, whereas 70 gene transcripts were upregulated in the uninoculated leaves (Schenk et al. 2003).

The transcriptomes of plants studied by using microarrays have yielded valuable insights into the stress-responsive genes found in these organisms. Additional genomics, proteomics, and metabolomics research are required to shed light on plant stress tolerance processes at the cellular level and better understand how genes, proteins, and metabolites interact to make healthy plant cells.

11.8 Advantages and Disadvantages of Microarrays

There has been a steady rise in the applications of microarrays due to its ease, lack of dependence on large-scale DNA sequencing, and ability to evaluate thousands of genes from numerous samples simultaneously. DNA microarrays can also be used to evaluate large-scale DNA variants, rapidly investigate chromosomal structure, and locate protein-binding areas on DNA. The advantages of microarrays over Western and ELISA analyses include increased throughput, multiplex analysis, reduced reagent consumption, high sensitivity, and fewer samples needed.

High costs, many low-specificity probes, and a lack of control over the transcript pool are some of the significant microarray restrictions. The most frequently used microarray platforms can only employ one set of probes designed by the manufacturer (Everett et al. 2010).

11.9 Microarray Costs in Comparison

Microarrays have been criticized for being prohibitively expensive for frequent use. However, as sales volume and automation in manufacturing increase, costs of molecular equipment decrease. For example, the price of qPCR machines has dropped by 90% since their invention a decade ago. Clondiag (http://www.biochipnet.com/node/154) and other low-cost microarray systems use a reader technology that lowers expenses (Barrett et al. 2009). These microarray approaches have been compared to standard culturing and biochemical methods for bacterial identification and found to be superior and less labor-intensive. In contrast, qPCR is

labor-intensive and expensive due to the high cost of reagents and the need for multiple runs to identify a wide variety of species reliably. It is still prohibitive for regular diagnostics to employ next-generation sequencing because of the high prices of next-generation sequencing, even as the technology improves. Selective gene regions that can be used for identification in these molecular diagnostic techniques are the most expensive (Arvanitis et al. 2014). The development of diagnostic oligonucleotides necessitates an international collaboration. The expenses will fall even further when gene and genome sequences are made publicly available.

11.10 Future Perspectives

The current chapter has summarized the various aspects of microarray technology employment in the field of molecular plant pathology. The microarrays will directly impact the agriculture community once the cost and user-friendly device developments become within reach for use by the diagnosticians and farming-related services. Moreover, microarrays will continue to be utilized to explore the diversity of microbial communities, even as next-generation sequencing becomes more widely available. Microarrays allow for the relative quantification of differences among samples. As a result of their large capacity for multiplexing, microarrays still have many advantages over other diagnostic techniques. The difficulty and length of time needed in creating and testing genomic sections that identify one species from another are a key hurdle to the commercialization and future development of microarrays for organism identification. Other molecular identification methods, like as sequencing, are also hindered by a lack of discriminating information. If current trends continue, microarrays may become a common diagnostic tool for plants, humans, and animals. Microarrays may be replaced with smaller biosensors when a single pathogen or a few pathogens are stored on an electronic platform. Electromagnetically charged magnetic beads might be used to produce target-probe complexes, which may be monitored by ultrasensitive thin-film sensors with magnetic fields holding an electric charge. Detection of one pathogen DNA molecule should be possible in a short amount of time using the sensor.

A transistor network has recently been used to identify DNA point mutations electronically (Pouthas et al. 2004). An antibody tagged with a luminous dye could be used to detect markers associated with a specific disease. A fluorimeter can see fluorescence quenching due to proper antigen binding in this scenario. The deployment of these devices in the battle against bioterrorism would be enormously beneficial (Mattmann 2020; Zahavy et al. 2003). These kinds of sensors have been developed by a wide range of companies and research institutions. However, the cost of microarray technology will continue to diminish with innovations in easy-to-use platforms, which would directly impact plant disease mitigation through accurate pathogen diagnosis and understanding of the cellular mechanisms underlying disease development.

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Predictive Models for Plant Disease Assessment

12

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Abstract

The world's increasing population demands more food commodities to fulfil the nutritional requirements. Increasing cultivation area and crop production and reducing yield losses will be required to meet food demand. Plants in gardens, forests, production fields, and landscapes are subjected to numerous health problems that cause yield reduction. Disease negativity in yield loss can be tackled by adopting appropriate management practices. Plant disease management requires diagnosis and severity assessment. Plant disease modeling is an important field for assessing the intensity or severity of the disease. It is a management system enabled to forecast the occurrence and any change in severity or intensity of plant diseases. Forecasting the occurrence of diseases under a specific area and time; however, appropriate preventive and control measures can be taken in advance to obtain potential yield. Applying management practices at the appropriate time reduces the wastage of crops and chemicals by forecasting disease and making it cost-effective. Basic components of plant disease are needed to investigate to make disease forecasting schemes. There are seven basic requirements for successful disease prediction. Intensity, incidence, and severity are primary disease measuring terms used to study disease forecasting. Modeling involves field observations, disease measurements, and weather conditions favoring disease spread, mathematical formulas, and computer use. The mathematical and analytical relationship gives information about interaction among the host, pathogen, and weather variables in mathematical equations presented as simple statements, tables, or graphs. Disease prediction can be made based on parameters involved, i.e., inoculum, weather variables, and comparative information. Computer simulation is beneficial for growers to

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understand the effect of components and subcomponents of the epidemic on yield loss. Many prediction schemes have been published.

Keywords

Plant disease forecasting · Elements of plant disease prediction · Computer simulation · Disease predictive models

12.1 Introduction

The tremendous increase in global population requires a large agriculture production to fulfil food demand (Kitzes et al. 2008; FAO 2009). Production, availability, nutritional status, and natural ecosystem protection can be ensured by using modern farming procedures (Kamilaris and Prenafeta 2018). Agricultural production must be two-fold by 2050 to meet the food demand of the increasing population pressure. Precision agriculture is the only way to improve productivity by using resources efficiently under different challenges of farming, such as agricultural land degradation, drastic climate change, urbanization, high input costs, and yield losses due to diseases. The health challenges of plants can be addressed by adopting smart farming (Tyagi 2016), including sensing technologies that indicate the growth and health of crops and ensure food security and sustainability (Gebbers and Adamchuk 2010; Narvaez et al. 2017). Climate change creates favorable circumstances for plant diseases. Crop diseases exhibit a promising role in reducing agricultural productivity by causing 20–40% losses and increasing the risk of food insecurity (FAO 2017). Proper and timely applied disease management strategies will help meet the food demand of the population by 2050 (FAO 2011). Several scientists have researched how weather and environmental conditions affect plant disease development (Rong et al. 2015; Das et al. 2016; Newberry et al. 2016; Donatelli et al. 2017; Raza et al. 2019; Fenu and Malloci 2020). Continuous monitoring, measuring, and analysis of various physical aspects and phenomena require to understanding complex and unpredictable ecosystem of agriculture through the application of information and communication technologies (Internet of Things) (Boursianis et al. 2020), remote sensing (Weiss et al. 2020), and cloud computing (Mekala and Viswanathan 2017). These technologies contribute to managing special and temporal changes for improving crop production and environmental quality (Pierce and Nowak 1999), producing massive agricultural big data. Agriculture big data is helpful in yield prediction (Chlingaryan et al. 2018), crop and food detection (Fu et al. 2018), weed detection, pest/disease detection (Milioto et al. 2018), food safety (Cheema and Khan 2019), risk management and equipment management, and also plant and disease prediction (Fenu and Malloci 2019, 2020, 2021). The recent advancement in the analysis of agricultural big data is through machine learning (ML) (Liakos et al. 2018), artificial intelligence (Fenu and Malloci 2020), and deep learning (Kamilaris and Prenafeta 2018). Crop disease management using advanced strategies is a need of the hour to secure the wastage of agricultural production.

Plant–pathogen interaction under conducive conditions, sometimes disease may occur in epidemic form. Most epidemics cause minor losses as controlled naturally, and occasionally, severe epidemics become out of control and can cause massive yield losses, ultimately damaging the economy (Ul Haq and Ijaz 2020).

Diseases are being controlled by several methods: cultural (crop rotation, sanitation, etc.), biological control, and chemical control (chemicals may use for foliar spray, soil sterilization, seed treatments, and soil amendments). Chemicals are important for the immediate control of plant pathogens. Due to the more effectiveness of chemicals over other control methods, grower reliance has increased. But hazardous effects of pesticides by judicial use on the environment and consumer's health must restrict their too much application. Reduction in use of chemicals can be achieved by the forecast when the crop is to be at economic threshold level and under which environmental conditions application of pesticide is practical to avoid wastage.

The foremost reasons for disease forecasting are economic, health, and environmental safety and appropriate use. The timely application of pesticides reduces waste as applied only when required, making it economically effective. Some countries have a target to reduce the use of pesticides (Jorgenson and Secher 1996). Consumer demand for pesticide-free crops can be fulfilled and justified using forecasting schemes. Plant disease forecasting is the main part of applied epidemiology and is also pivotal to management practices.

This chapter covers the promising aspects of plant disease forecasting, including the introduction, fundamental elements of plant disease prediction (host, pathogen, environmental conditions), requirements of prediction, measurement of disease, data for prediction, modeling of disease, challenges, methods for disease prediction, computer simulation, and prediction scheme and models.

12.2 Plant Disease Forecasting

Prediction of plant diseases means forecasting the occurrence of diseases under a specific area and time; however, appropriate preventive and control measures can be taken in advance to obtain potential yield. It is a management system enabled to forecast the occurrence and any change in severity or intensity of plant diseases. Predictions of probable disease outbreak and increase in disease severity involve an organized working team and expenditure of energy, money, and time. It is used as an aid to the timely implication of management practices. Prediction forecasts the conditions for establishing the pathogen when the susceptible host is available. The disease predicting scheme requires a complete understanding of the epidemiology and etiology of the pathogens and diseases. It is a cost-effective method of management by providing information about when and where disease management practices must be required and enabling the person to avoid unnecessary operations. It helps evaluate the input costs and benefits. The use of chemicals can also be reduced by an effective warning system based on the accuracy of the prediction model (Sigvald 2010). Forecasting the disease outbreak increases profitability and lessens the negative impact of diseases on fauna and flora, as disease prediction

indicates when the particular disease becomes epidemic form or remains with less intensity and whether chemicals would be applied or not. Forecasting is highly valuable in making wise economic decisions (disease forecasting chapter).

12.3 Fundamental Elements of Plant Disease Prediction

The occurrence of plant disease is highly dependent upon weather fluctuations. The disease will occur if a susceptible host, virulent pathogen, and suitable environment are present simultaneously (Fig. 12.1). The disease triangle is considered a fundamental principle of the components involved in plant disease causation. Three components of the disease triangle (Fig. 12.2) are host, plant, and environment,

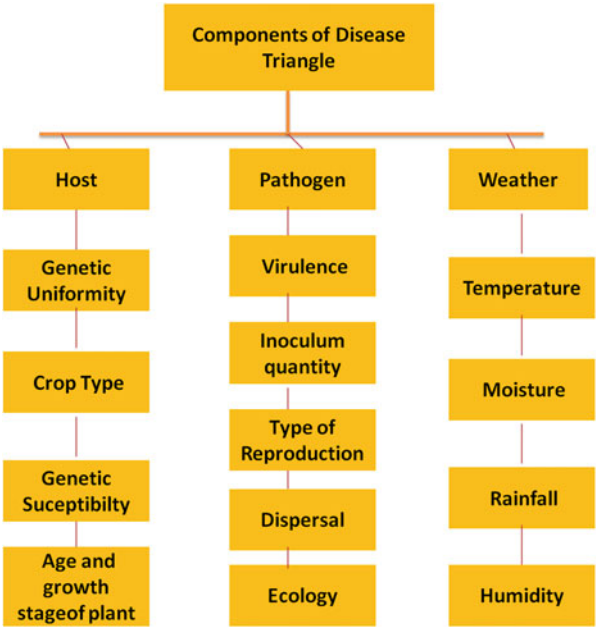

Fig. 12.1 Various factors of host, pathogen, and environment involved in epidemic

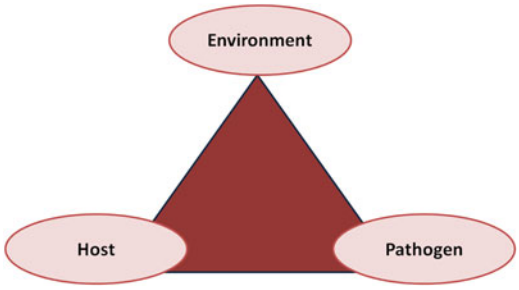

Fig. 12.2 Disease triangle representing three basic components of plant disease epidemics

and the disease triangle drawing was first time published by (Stevens 1960). Afterward, more parameters were added to the basic concept of disease development, i.e., time and human activity (Francl 2001; Agrios 1988; Zadoks et al. 1974).

12.3.1 Host

Many external and internal factors of the host plant affect the development of plant disease epidemics. Host resistance and susceptibility determine the speed of disease development. Host plants have two main types of resistance, i.e., horizontal and vertical. The pathogen cannot be established in plants carrying vertical resistance while plants become infected in case of plants have genes for horizontal resistance. Resistance genes are absent in a susceptible host, which is ideal for establishing new infection by a pathogen. The interaction of pathogen and host and disease development may be considerably affected by the age of the host plant. Resistance changes with the age of the host plant and growth stages, such as the host plant of peach leaf curl, bacterial blights, and systemic smuts; rusts are susceptible during the growth period and resistant at the adult stage. Epidemics develop more rapidly in annual crops (vegetables, corn, rice, cotton, and fruit diseases of trees and vines) than in perennial crops (pear decline, Dutch elm disease, and chestnut blight take many years to develop).

12.3.2 Pathogen/Inoculum

Highly virulent pathogens can infect the plant more rapidly and are responsible for large amounts of primery or secondary inoculum. Suppose if a large inoculum is present near the host plants; there will be more chances of epidemics. All the pathogens are categorized based on their life cycle as monocyclic or polycyclic. Most of the plant pathogens can produce many offsprings with a short reproduction cycle known as polycyclic (has many generations in a single growing season). While some pathogens produce less offsprings (inoculum) by completing one reproduction cycle in a single growing season are monocyclic. The mode of dispersal (air, soil, rain splashes, water) of the pathogen/inoculum significantly affects the disease and epidemic development (Agrios 2005). Polycyclic pathogens (fungi causing rusts, mildews, and leaf spots) produce more inoculum, so dispersal will also be high and cause widespread epidemics due to the causation of a series of infections as compared to monocyclic pathogens (cedar-apple rust-2 years, dwarf mistletoe-5–6 years) as lesser the inoculum production limits the dispersal and fewer chances of disease spread.

12.3.3 Environment

The environment is considered one of the driving forces for plant disease development. Major climatic components include temperature, humidity, rainfall, leaf moisture, and solarization. Environment affects the host plant's growth stage, succulence, susceptibility, and availability. The environment may also hugely affect almost all activities pathogen such as survival, multiplication, germination, sporulation, dispersal, and penetration. The number and activity of pathogen vectors may be driven by temperature. Environment plays a pivotal role in epidemiology and forecasting a particular disease. The duration of environmental events primarily affects epidemiology. Accurate meteorological data is required for disease prediction models (Agrios 2005).

12.4 Requirements for Disease Prediction

The basic requirements for successful disease prediction are:

1. The disease must cause economically considerable loss in yield and quality. Damage assessment is necessary for the development of management strategies.
2. The disease progression rate varies from initial infection to later stages. If it is not, there will be no need for disease forecasting.
3. Management measures must be readily available at an economically affordable cost.
4. The prediction criteria must be based on investigational research carried out in the series (in vitro, in vivo) to establish its applicability.
5. Growers must have the equipment and enough human resources for application management measures at the time of disease warning.
6. Weather information concerning disease must know.
7. Warnings should be long term instead of short term.

12.5 Assessment/Measurement of Disease for Forecasting

There are different methods to measure plant diseases to estimate incidence, intensity, severity, and prevalence (Bock et al. 2010). The important terms in disease measurement and their interpretation are redefined according to the advances in this field (Madden et al. 2007). Disease incidence is a comparatively easy and quick method to estimate disease measurement and yield loss; it is commonly used in epidemiological and disease forecasting studies. In fruit diseases, disease incidence directly relates to yield loss.

Some basic measurement terms are as follows:

12.5.1 Disease Intensity

It is generally used to express the amount of disease in a particular population (Nutter et al. 1991).

12.5.2 Disease Incidence

Disease incidence is the percentage of diseased plants out of total plants observed (Nutter et al. 1991; Madden et al. 2007).

12.5.3 Disease Severity

The sampling unit's relative area exhibits disease symptoms (Nutter et al. 1991).

12.5.4 Disease Prevalence

Disease prevalence is the percentage or proportion of fields and counties in which the disease reveals the disease at a larger scale than incidence (Nutter et al. 1991).

12.6 Data for Plant Disease Prediction

Data for plant disease prediction can be obtained from many heterogeneous sources such as field surveys, field sensors, remote sensing (Duarte-Carvajalino et al. 2018), weather stations (Fenu and Malloci 2019), and pictures originating from hyperspectral cameras and web services (Sannakki et al. 2013).

12.7 Modeling of Plant Disease

Epidemic starts from a few plants, and its spread depends upon many factors such as type, magnitude, and prevailing duration of specific environmental factors. These factors drive the severity and spread of disease over a larger area. Epidemics come to a stop when all the host plants become dead, or weather conditions become unfavorable. The interaction of host, pathogen, and environmental conditions is required to understand the epidemics. A study about the interrelation of factors involved in epidemics helps make disease prediction models. Field observations, disease measurements, weather conditions favoring disease spread, mathematical formulas, and computer use are needed for prediction models. More of the variables involved in prediction models will be the accuracy rate. Validation of the prediction model is a significant research area in successful disease forecasting practices and adopting management measures. Model validation needs a series of experiments to determine

the difference between actual and predicted situations. Database (including information about the crop, pathogen, disease, sensors, and weather station location) is developed for constructing the disease prediction model. The mathematical and analytical relationship gives information about interaction among the host, pathogen, and weather variables in the form of mathematical equations presented as simple statements, tables, or graphs.

12.8 Challenges

Some crucial challenges have been articulated for advancing models by Newberry et al. (2016):

1. Flexibility and accuracy of a particular model.
2. Assumptions about contact and interaction and their statistical representation.
3. How to define critical or economic threshold under several infection recognition.
4. How to model the disease parameters that depend upon long-distance interaction.
5. Identify the operational resolution as a natural scale for modeling and interaction.

12.9 Methods for Disease Prediction

Plant disease forecasting models can be grouped into three classes based on input parameters.

- Disease prediction based on inoculum.
- Disease prediction based on weather conditions.
- Disease prediction based on comparative information.

12.9.1 Disease Prediction Based on Inoculum

Field history regarding the crop cultivated and the presence of the primary and secondary inoculum, its quantity, and viability are being determined to predict the particular plant disease's prevalence, intensity, and duration. The aforementioned pathogen characteristics impact the disease epidemic and are important for plant disease prediction models. If a large inoculum is present in or near the field, there will be more chances of disease spreading, so a management plan should be prepared before time. The timely application of control measures can prevent yield losses and increase output. Different methods are available to determine the inoculum's presence, quantity, and viability. Inoculum present in soil can be assessed by the monoculture method. Air trapping devices capture airborne spores and seed testing methods to determine inoculum in seeds (ergot of pearl-millet, loose smut of wheat, and viral diseases).

Erwinia stewartii causes Stewart's wilt in corn to survive in the body of the corn flea beetle (vector) during winter; if the number of vectors is more in the previous season, there will be a considerable quantity of inoculum present, and the chances of infection will be increased.

Fire blight disease of pear and apple caused by *Erwinia amylovora,* rate of pathogen multiplication reduces at below 15 °C temperature compared to temperature more than 17 °C. Inoculum quantity will be increased whenever the temperature exceeds the prediction line. There will be a need to apply bactericide to prevent an epidemic.

12.9.2 Disease Prediction Based on Weather Conditions

Weather is a driving force in plant disease epidemics; therefore pivotal role in disease forecasting, so that record of weather conditions is necessary. It includes temperature, rainfall, relative humidity, wind speed, cloudiness, and light duration and intensity to be recorded. Traditionally, mechanical equipment was used to measure all weather variables, and data record was maintained manually on chart papers as ink traces. But now, various electronic sensors are present and quickly produce electrical records. Computerized sensors are also available to improve measuring weather conditions for facilitating disease prediction. In some countries, battery operating electrical instruments measure environmental components. Thermometers, thermocouples, hygrothermographs, and thermistors (semiconductors-electrical resistance changes with temperature) are temperature measuring instruments, hygrothermograph for relative humidity, and string-type sensors for leaf wetness (slacken on dry and constrict when moistened). Electrical sensors can be placed on the leaf surface or among the leaves to detect the rain's moisture, dew, and duration. Wind, cloudiness, and rain are still detected or measured using conventional instruments, i.e., rain funnels, cup anemometers for wind speed, and wind direction by vanes.

In modern monitoring systems of weather factors, electrical sensors are connected to data devices to read on the digital display and transmit to cassette and printer. Cassette recorded data may be transformed to microcomputers, viewed, processed in computer languages, arranged by separating each variable, and plotted and analyzed. Accurate information about weather factors leads to an effective disease predictive model that provides the basis for sporulation and establishment of the pathogen; therefore, timely management practices can be adopted.

Various crop or plant diseases occur due to prevailing weather conditions (Fenu and Malloci 2019). Some researchers provide a solution for disease control based on analytical models (Berger 1989). Disease processes' complexity and dependence on various factors limit forecasting effectiveness (Bhagawati et al. 2015). Predictive models for growing rice and potato have been widely explained in literature (Katsantonis et al. 2017; Fenu and Malloci 2019). The online database (PestCast) from the University of California provides information for model development. Information and communication technologies (artificial intelligence techniques and

machine learning) are widely used in agriculture to discover and evaluate models to define complex mathematical relationships. Meteorological parameters-based forecasting may include advanced strategies, artificial neural networks, long short-term memory, support vector machines, random forest, extreme learning machines, deep learning machines, and multilayer perception to measure environmental variables (temperature, rainfall, humidity, wind speed, brightness) (Malicdem and Fernandez 2015), evaporation (Malicdem and Fernandez 2015; Xiao et al. 2018), and sunshine hours (Kim et al. 2018). It is preferred to process the weather data hourly rather than daily to make prediction models precise. Scientists investigated the environmental factors by collecting data on an hourly basis using the machine learning approach in Sardinia to predict the late blight of potato Fenu and Malloci. Data of temperature, speed, humidity, rainfall, and solar radiation were collected from various locations for four years and analyzed (Fenu and Malloci 2019). Support vector machine and feed-forward neural network were validated using a stratified cross algorithm (Fenu and Malloci 2020). The results revealed that temperature, wind speed, and humidity exhibited the primary role of the late-bight prediction model. Then decision support system used these models to help in decision-making.

Support vector machine and artificial neural network algorithms were used to predict rice blast disease. The incidence and severity of rice blast changes in a specific growth stage depend upon weather parameters during the same year. Results indicated that rainfall significantly impacts 48% of disease onset (Malicdem and Fernandez 2015). Region-specific prediction models were prepared for seventeen cultivars of potato. Temporal patterns of the environmental variables were considered during the modeling of the disease. Long short-term memory network was applied to past data (Kim et al. 2018). Nettleton et al. (2019) compared four different predicting models for modeling the rice blast disease. Modeling cotton diseases and pests have been done using modern techniques (Xiao et al. 2018). A combination of weather prediction and regression models was used for modeling the powdery mildew disease of grapes (weather variables mainly based on temperature and humidity) (Sannakki et al. 2013).

Artificial neural network was used as an analysis technique to classify and discriminate fungal infections of oil palm at early stages; results revealed that artificial neural network could recognize the relationships between environmental variables using spectral data and classify accurately (Ahmadi et al. 2017).

Warning models have been investigated for predicting potato late blight using unmanned aerial vehicles and captured 126 multi spectral images, deep learning, and machine learning algorithms (Duarte-Carvajalino et al. 2018). Remote sensing information is used as the data source for the powdery mildew prediction model (Zhang et al. 2014). The scientist identified the vegetative growth stages sensitive to powdery mildew to establish a predictive model based on regression analysis. Nine input variables were included in this model (Zhang et al. 2014). Pre-symptomatic identification has been demonstrated in the case of foliar disease of sugar beet; it is helpful to identify the diseased leaves from non-diseased leaves, differentiate and identify the conditions before, i.e., powdery mildew, leaf rust, and Cercospora leaf spot (Rumpf et al. 2010). Pre-symptomatic identification of tobacco diseases has

been investigated using hyperspectral imaging combined with back-propagation neural network, support vector machine, and extreme learning machine (Zhu et al. 2017).

12.9.3 Disease Prediction based on Comparative Information

Prediction about disease development and epidemics, criteria developed from various comparisons of disease observations with standard environmental data being provided for diseases. Meteorological data of past years are collected from different sources and correlated with the intensity of the plant diseases. Then, the data is compared and forecasted the plant diseases.

12.10 Computer Simulation

Computer simulation is beneficial for growers to understand the effect of components and subcomponents of an epidemic on yield loss. It is helpful in the actual situation of the disease. Computer simulations help evaluate the importance or role of each epidemic subcomponent at a specific duration in terms of yield loss. Computer simulations provide attention to effective control measures against specific subcomponents of an epidemic by highlighting the subcomponents of an epidemic. Computer simulations effectively evaluate the disease status (Table 12.1) and the efficiency of applied control measures. Computer simulations have various benefits as they provide information about disease spread and severity with time and also about economic losses.

Several computer simulation programs are readily available. EPIDEM is the first written computer simulation program designed for the early blight of potato and tomato disease caused by Alternaria solani. It was written in 1969 and obtained from modeling of the life cycle of Alternaria solani.

Table 12.1 Important computer simulators along with pathogens/diseases

Sr. No.	Computer simulators	Pathogen/disease
1	EPIDEM	Early blight of potato and tomato
2	CERCOS	Cercospora blight of celery
3	MYCOS	*Mycosphaerella* Blight of Chrysanthemums
4	EPICORN	*Cochliobolus maydis*
5	EPIVEN	Apple scab caused by *Venturia inaequalis*
6	EPIDEMIC[a]	Stripe rust of wheat

[a]EPIDEMIC-it is highly flexible and general (modification can be done quickly)

12.11 Prediction Scheme and Models

The models interpret or represent the conditions that affect the pathogen's development, dispersal, survival, and infection capability. There is an unlimited variety of various combinations. Advance computers can solve different technical constraints. Biological processes involve changes such as new strains evolve; therefore, aggressiveness and adaptation of temperatures fluctuate may be covered by most of the models.

Several scientists have published forecasting schemes globally on various pathogens and crops. Potato is an important crop sprayed many times against late blight disease (Garthwaite et al. 2002). The forecasting scheme for late blight disease greatly depends upon computer processing and modeling. The disease progress curve is a fundamental component of forecasting the disease. Disease to yield loss relationships can be assessed by the disease progress curve (Large 1952). Potato late blight disease is one of the destructive diseases as it causes losses in yield and store conditions. Potato growers applied up to fifteen sprays in a growing season (Hardwick and Turner 1996). Due to the importance of the potato crop, many forecasting schemes have been constructed. The interval between spray applications becomes reduced as the season progresses, indicating blight is controlled by protectants rather than eradicants. Disease modeling help to know about the application of spray at the right time (Large 1959). Pathogen eradication is possible with the development of phenylamide-based fungicides (Urech et al. 1977). Phenylamide-based fungicides act as eradicant and are systemic. Its efficiency becomes cease with the resistance development in pathogen (Cooke 1992). Later developed fungicides application in the absence of late blight disease negatively affects yield (Taylor et al. 2000).

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Extension Plant Pathology

13

Muhammad Atiq and Nasir Ahmed Rajput

Abstract

Phyto health care is a holistic approach and the most pressing need to serve the poverty-stricken farmers by extension services, especially in developing countries. Extension programs can prove fruitful tools to minimize plant disease losses by assisting and educating farmers in developing countries in order to increase their productivity, food security, and livelihoods. Phyto health clinics are accountable and responsive. Previous studies have revealed significant lessons about the most effective ways to set up phyto healthcare clinics, educate phyto doctors to provide a trustworthy service to farmers, and lay the groundwork for developing plant health systems that integrate efforts and resources for increased impact on farmers' economic status. The participatory action research approach has made it possible for us to pinpoint research priorities precisely and has given us a better understanding of the constraints faced by farmers. The main aim of this effort is to throw light on the significance of phytopathology extension, the role of plant pathologists in phyto healthcare, and an overview of phyto healthcare in the developed and developing world.

Keywords

Plant health · Pathological issues · Plant health diagnostic network · Phyto clinic · Global biosecurity

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13.1 Phyto Healthcare for Poverty-Stricken Farmers Across the Globe: A Pressing Need Extension Plant Pathology

Extension plant pathology aims to build educational programs that offer guidance on prompt detection and management of plant disease to increase farmers' productivity, food security, and means of subsistence.

Programs for extension are required to help increase farm income and production and decrease food insecurity and poverty. Extension programs involve teaching techniques and skills to improve farmers' productivity, food security, and means of subsistence. In agricultural-dependent economies, extension programs have been the primary means of disseminating knowledge on-farm technology, promoting adult learning in rural areas, and helping farmers to improve their managerial and technical farm skills. With a focus on the agricultural extension services offered by the ACDEP, the impact of extension services on-farm production and income has been investigated (Danso-Abbeam et al. 2018).

Since the Smith-Lever Act of 1914, which established extension, it has significantly changed (Sherf 1973). It deals with extension programs designed to minimize plant disease losses by assisting and educating county extension agents and producers through the county delivery system. Early outreach, a hallmark of extension plant pathology, is described as a one-way process in which the institution communicates its knowledge to crucial elements (Spanier et al. 2001). Over the past century, changes have also been made in the diversity of the network that shares plant pathology knowledge. Growers rely on the diagnostic services and adaptive research carried out by extension plant pathologists and information from non-profit organizations, for-profit companies, and paid consultants. Companies, associations, or consultancies rarely do independent research. The Rodale Institute and the agricultural chemical sector stand out. However, most of these sources of information have a narrow scope because they concentrate on a specific set of issues or strategies. For instance, non-profit organic organizations can't even teach growers how to use a genetically engineered disease-resistant cultivar, even if doing so is economically and environmentally advantageous. Agrichemical suppliers who sell specialized chemicals frequently offer advisory services to growers (Everts et al. 2012; Ul Haq et al. 2020).

13.1.1 Extension Plant Pathologists

Plant pathologists who work in extension programs recognize plant diseases, offer guidance, and conduct research on regional and local phytopathological problems. Although the job of an extension plant pathologist has evolved through time, the need to be knowledgeable about every facet of our profession and be able to apply and successfully communicate that information to a broad audience does not appear to have altered. The purpose of extension has always been to act as a "translator" between the university and its customers, including nurserymen, agribusiness owners, farmers, homeowners, consultants, and a wide range of other people. The

first officially hired extension plant pathologists were M. F. Barrus of Cornell and R. E. Vaughan of Wisconsin in 1915. Extension plant pathologists planned and conducted short courses, farmer institutes, and local grower meetings during this time. Plant pathologists have already begun working on extension publications and demonstration initiatives. Extension plant pathologists eventually coordinated their work through county agents as the county agent system grew more established. County agents were responsible for communicating the local community's requirements to state-based extension specialists (Jacobsen and Paulus 1990).

13.1.2 Need for Extension Phytopathology

If not addressed, there would be a delay in responding to developing diseases, lower crop yields, more significant economic losses, and a disadvantage for US agriculture on the global stage due to the increasing demand for reliable information and continual budget cuts at the local, state, and federal levels (Everts et al. 2012). Over the past 20 years, numerous extension plant pathologists have worked at regional or extended research centers. However, the APS extension committee met for the first time in 1931.

13.1.3 Scope of Extension Phytopathology

Extension phytopathology is bringing scientific investigations to the public. Extension plant pathologists are still an essential part of the APS, accounting for 9% of the organization's membership in the United States (Jacobsen and Paulus 1990). Extension plant pathology programs have changed throughout the past century to account for changes in program funding and the US population's demographics. In response to a highly educated customer, extension programs are becoming more collaborative and specialized (Everts et al. 2012).

13.1.4 An Overview of Plant Healthcare in Developed and Developing Countries

Despite a recent concern for extension, a dearth of innovation in advising approaches has resulted from historical neglect. However, in developing countries, extension programs perform a terrible job of aiding farmers. Although governments employ a substantial number of extension specialists, they have a limited understanding of farmers' needs, and farming communities are scattered (Anderson 2007). Because farmers have few steady and trustworthy channels for communicating their requirements, even the most excellent extension workers cannot meet the demands of all their assigned customers. It is time to reevaluate how to best address the requirements of farmers, especially in developing nations where agriculture serves as the primary source of earnings for millions of people.

Phyto health clinics are not a novel concept in the USA and other developed regions of the world with successful phyto health systems that help farmers and have been doing so for a long time (Campbell et al. 1999). However, in the case of the developing world, phyto clinics present a new strategy for the farming community. The most pressing need is to serve the farmers by extension services, especially in developing countries. Extension programs can prove fruitful tools to minimize plant disease losses by assisting and educating farmers in developing countries to increase their productivity, food security, and livelihood.

13.2 An Insight into Participatory Approaches and Phytopathological Problems of Developing Countries

The use of pesticides imposed concerns on growers, agricultural workers, and users, and the centralized strategy came under increasing criticism. A new paradigm was needed to feed the world through sustainable food production (Schillhorn van Veen 2003). The centralized system (including state-managed input procurement) came under increasing criticism since the usage of pesticides posed risks to farmers, agricultural workers, and consumers. A new paradigm was needed to feed the world through sustainable food production (Schillhorn van Veen 2003). Farmers today must compete in markets where consumers expect low-cost and high-quality products. Pests (such as diseases and weeds) are becoming more capable of adapting to cutting-edge single technology-style crop protection and contributing to the problem. There is a need for holistic approaches to generating and sharing knowledge (Vos et al. 2010). Participatory methods are currently utilized more frequently, and several words and acronyms refer to various uses. The shared denominators include the emphasis on the target/end-user groups' active involvement in the discovery of innovative knowledge and the facilitatory contribution of trainers.

13.3 Emerging Plant Diseases in Developing Countries

Plant diseases significantly lower crop yield in tropical ecosystems and developing countries. These countries are especially vulnerable to spreading diseases because they lack the resources to control them. Major epidemics can substantially harm economies that depend heavily on agriculture, jeopardize national food security, and evict populations. According to current forecasts, cropping practices and international trade will lead to an increase in disease pressure. A detrimental factor might also be climate change. Ideal strategies for containing outbreaks should include prompt intervention, precise detection, and reliable monitoring. This is especially true for new diseases, which frequently need rapid development and innovative responses. Emerging infectious diseases spread by plant pathogens have the potential to develop into sudden and severe epidemics due to the interaction of various pathogen, host, and environmental variables. In the past, Europe endured

catastrophic epidemics with adverse societal effects due to the continent's rapid urbanization and the fact that many other EIDs still regularly occur in the developing world.

At the start of the twenty-first century, Xanthomonas Wilt of Banana, which threatened the food security of nearly 70 million people in Uganda, caused yield losses of about 50%. (Vurro et al. 2010). The damaged area is expected to grow at an annual pace of 8% if the disease is not controlled (Kayobyo et al. 2005). According to predictions, the condition will cost at least $8 billion in damages over the next ten years or $2 billion yearly. Current research indicates that Uganda's banana crop will endure output reductions of about 53% during the next ten years. Cassava Mosaic Virus (CMV) is the most severe disease in Sri Lanka, Southern India, and Africa (Otim-Nape and Thresh 2006). CMD is a disease that can eradicate cassava farming in many parts of East Africa and lower yield by 80–90%. In Sub-Saharan Africa, a parasitic weed called *Striga hermonthica* destroys grain crops on about five million hectares (Vurro et al. 2010). Infestation rates in maize fields ranged from 20 to 30 percent of the total in Togo, Mali, and Nigeria, and Benin exceeded 65 percent in a review on the spread of *S. hermonthica* in 25 African countries (De Groote et al. 2008).

Due to the *Puccinia graminis* f. sp. *tritici* race Ug99 rust fungus, which adversely affects wheat in Uganda, most nations that farm wheat are in danger. A race known as Ug99, named after its discovery in Uganda in 1999, has recently produced severe outbreaks in several countries in East Africa and the region surrounding the Horn of Africa (Ethiopia, Kenya, Sudan, Uganda). Oman, Iran, Eritrea, Afghanistan, and Pakistan (FAO 2008b) were on the list of nations with an immediate risk of infection.

13.4 Participatory Methods

There are many applications for participatory approaches and numerous terms and acronyms to identify them.

13.4.1 Adopting Participatory Training

It is advantageous when information is available, whether at the researcher or farming community level. Such information is disseminated through problem-solving instruction that promotes the discovery learning process, which entails diagnosing or identifying the problem, researching the ecology and biology of pests, and then trying out various management techniques. During participative training, conventional teaching techniques must be abandoned. We refer to them as participants rather than trainees or students. In the past, farmers could observe tomato plants suffering from wilting in their fields but did not execute rouging because they were unaware that infected plants could spread disease to other plants. Farmers engaged in a field exercise where they performed the customary task of

harvesting infected plants at the base of the stem and placed a portion of the infected stem into a glass containing water to detect the bacterial ooze (Vos et al. 2010).

13.4.2 Participatory Research

Participatory research may be helpful when conventional wisdom and scientific counsel clash or when there are no effective solutions to farmers' concerns. There are several definitions, but broadly speaking, the farming community designs agenda, analyses, and develops technologies following personal needs with the help of enablers and resource people.

There are various definitions, but generally speaking, with the help of facilitators and resource people, farmers determine the agenda, conduct assessments, and develop technologies according to their needs. Farmers must take the initiative and participate more actively in the field activities because the results are unknown, even if there are few procedural distinctions between participatory training and participatory research (Vos et al. 2010). Although direct empowerment is frequently restricted to small farmers when conducted by research groups, participatory research can also lead to farmer empowerment (Hellin et al. 2008). On organic smallholdings, a collaborative assessment of biological and cultural management looked at seven biological treatments utilizing a variety of *Clonostachys* and *Trichoderma* species and weekly and monthly phytosanitation. For the best results, farmers choose a mix of cultural and biological control. Because there was no alternative method for biocontrol at the time, weekly phytosanitation was still necessary. However, with appropriate biocontrol, pod sporulation can be avoided, and the phytosanitary process is becoming less labor-intensive (Vos et al. 2010).

13.5 Impact Assessment

A collaborative effort between scientists and farmers can also be used for impact assessment. Farmers should be urged to maintain their records in order to aid this process. Farmers will express interest in continuing the activity in their worksheets once they see how important keeping notes is. The breadth of FPR's coverage has drawn criticism (Farrington 1998). The recommendation domain is used to identify the population sample and to create a plan for maximizing the advantages of farmer involvement in events (Conroy and Sutherland 2004).

Adopting participatory approaches requires adopting prescribed practices and methods, making their successful implementation a difficult task. At present, extension workers must interact with numerous sectors, each with its own institutional and personal history, conventions, values, and interests. Farmers will come to know about plant diseases and management strategies. It will prove a beneficial step for effective learning of farmers.

13.6 Technology Espousal: Farmers' Participation and Training in Pakistan

Pakistan is an agriculture-based country. Most Pakistani citizens, whether directly or indirectly, depend on agriculture for their daily needs. The Pakistani government has begun several initiatives to educate farmers (Iqbal et al. 2016) and to provide food supply, combat poverty, and access services such as education and healthcare. Smallholders must be able to generate sustainable income. Sustainable productivity lessens the negative impacts on the environment and natural resources. The delayed adoption of modern technology in Pakistan is the most significant barrier to better smallholder yields.

13.6.1 The Cotton Industry in Pakistan and Constraints Faced by Farmers

The country's backbone is cotton. Over 1.5 million farming families are employed, while millions of workers in cities and towns depend on it for their livelihood. Up to 40% of the financial income of rural households in cotton-growing regions comes from selling cotton-related products. In addition, it generates around 85% of our domestic oil production and 60% of our export revenue. The province of Baluchistan has become a significant region for cotton production in addition to the central cotton-growing areas of the Punjab and Sindh provinces (Baluch 2007). Boll rots, root rot, and wilts are the three main fungal diseases. Bacterial blight, caused by *Xanthomonas campestris* pv. *malvacearum* are the most ravaging phytopathological issue. Bacterial blight is the most harmful disease that significantly reduces cotton yield during the rainy season (Delannoy et al. 2005). The United States' state of Alabama was where it was initially documented in 1891 (Atkinson 1891). The most notable factor limiting cotton output in Pakistan is CLCuD (Sattar et al. 2017). A new challenge to cotton growers is cotton boll rot and bacterial seed with 10–15% yield loss (Hudson 2000). Many fungal species cause cotton seed rots and seedling infections. Pythium (Devay et al. 1982), Rhizoctonia (Brown and McCarter 1976), Fusarium (Klich 1986), and Thielaviopsis are the main fungal genera linked to seed degradation (King and Barker 1934). These infections infiltrate the vulnerable cotton plants and cause damping-off disease. Farmers' poor technical proficiency and the lack of a productive extension agency, which limits access to and dissemination of new information and technology, make these problems much worse (Guest et al. 2007).

13.7 Participatory Action Research

Farmers are both participants and researchers in participatory action research. The primary goal of PAR was to draw attention to troublesome situations or concerns that participants believed required more research to make effective practice

improvements (Cornwell 1999). The program aimed to provide farmers with efficient disease management solutions. The idea that PAR provides various valuable and affordable solutions is one of its advantages.

The results of the several research that examined the effects of Bt cotton in developing countries are generally restricted to comparing the average outcomes for adopters and non-adopters. The findings of the several studies that looked at how Bt cotton affected developing economies were usually limited to a study of the average results for consumers and non-consumers. Concerns and questions have been raised about the commercial use of Bt cotton in Pakistan due to a lack of thorough research on the economic growth of the readily accessible Bt varieties relative to traditional types and a variety of pest risks in the country (specifically losses brought on by the CLCuV, which is dispersed through the white fly). To assess the financial impacts of the adoption of unapproved Bt varieties on costs of production and yields, the "Bt Cotton Survey 2009" (IDS 2009), a questionnaire-based survey, was conducted in 2009 in two cotton-growing districts of Pakistan: Mirpur Khas in Sindh and Bahawalpur in Punjab (Becker and Ichino 2002). Between 2006 and 2008, Bt cotton adoption grew quickly in these two districts, following the general trend. Bahawalpur had a higher adoption rate (36%) than Mirpur Khas in 2006 (32%). Bt cotton was grown by slightly more than 87% of farmers in Mirpur Khas in 2008, compared to 74% in Bahawalpur (Nazli 2011).

13.7.1 Significant Management Options for CLCuV

A variety of several minimal, moderate, and high-input IPDM solutions were introduced in response to the realization that management approaches needed to change. These alternatives served as the basis for contemporary, grower-focused methods of disseminating cutting-edge knowledge and methods (Konam et al. 2008). The objective was to increase cotton production and increase farmer incomes. By adjusting optimum crop techniques, the disease could also be controlled. The severity of the disease would be minimized by planting cotton between May 15 and June 15. CLCuV infection is reduced by about 20–30% by applying potassium fertilizer at 100 kg K2O and 150 kg N per hectare. In the cotton belt of Punjab province, disease prevalence varies substantially naturally depending on location (Baluch 2007). The participatory action research approach has made it possible for us to precisely pinpoint research priorities and better understand the challenges faced by farmers and what is and is not working. Because they were immediately involved in implementing the improved management and spreading it among their neighbors, it gave farmers more responsibility by demonstrating the immediate consequences of technology.

13.8 SoTL Projects in Phytopathology

Teaching efforts valued on par with scholarly activity, such as research in our professions is the aim of those who promote scholarship learning and teaching. When a teacher faces a severe issue, it is typically considered a negative situation that must be addressed with caution (Bass 1999). A founding member of the movement for the scholarship for learning and teaching claimed that it is appropriate for a scientist to teach, trying to provide the fairest and most realistic description of the latest information to those who would like to secure knowledge, in addition to discovering the truth and sharing it with his colleagues. To become a scholarly teacher, one must reflect on their teaching and have a keen interest in learning more about the research behind effective teaching techniques. Such a method frequently entails articulating a problem, finding its solution, and performing a formative assessment to gauge its effectiveness (Bass 1999). A topic in education must be thoroughly investigated for SoTL (study of teaching and learning), and the results must be shared with people through presentations or publications. This knowledge might then be used to develop a framework for other teachers (Hutchings and Shulman 1999).

13.8.1 Scholarship of SoTL Projects in Our Classroom

As academic educators, we want to know if the materials we offer and the teaching strategies contribute to improving student learning (Eastburn and D'Arcy 2010). This inclusive classrooms phytopathology course provides an introduction; we shall present three SoTL investigations. Students of phytopathology learn about the main crop plants and the most significant diseases that affect them. Students are also addressed to agricultural issues such as mycotoxins, pesticides, genetic engineering, food safety, environmental quality, monoculture, and food production through the use of these plant diseases.

13.8.2 First Example: Productiveness of Various Media and Instructional Methods

We deliver material in a variety of instructional formats and media, including videos, small group activities, traditional lectures, books, websites, podcasts, response devices for students' in-class and out-of-class writing assignments, to accommodate variation and diversity of our students' majors, backgrounds, genders including learning styles (D'Arcy et al. 2007). Therefore, these media might be beneficial for a variety of student groups. Students from various majors, learning styles, and genders judged a range of teaching strategies and media as helpful in this study. Eight teaching techniques, such as PowerPoint slides, review grids, online quizzes,

lectures, overheads, PowerPoint notes, and blackboard notes, were evaluated as successful over the course of two or more semesters through each of the four learning style groups (scientific and non-science majors group and both genders group). Practically speaking, it is good that different student groups found these strategies helpful, and it supports the notion that various teaching strategies and media benefit all students (Eastburn and D'Arcy 2010).

13.8.3 Second Example: Effectiveness of Web-Based Assignments

A second study was carried out to determine the effect of a supplementary course website on student learning. Data from the student survey were analyzed, and seven key themes about student site usage emerged. More student control over information access, more learner speed control, and a wealth of knowledge resources are all provided via the internet. Practical learning of students is possible by writing, and they can explore the field through interactive components such as available online laboratories, including dynamic simulations, while the internet allows for in-depth research and phenomenon investigation (Bruce et al. 2005). We lacked any complex data showing that utilizing the site had enhanced student learning, even though input from students suggested they enjoyed using it and thought it aided their in-class learning. We were able to determine the impact of the productive activity on the learning of students through a comparison of results of comprehending and applying the scientific method pre-test questions to those on questions with a similar phrasing on the post-test. As a result, the potential of students to explain the scientific process's main ideas and apply what they learned to particular circumstances significantly improved.

13.8.4 Third Example: Significance of Course Information to Daily Lives of Students

As educators, our goal is for our students to understand the significance of what they learn in school and use it in their everyday lives. In the Plants, Pathogens, and People course, we undertook third research to learn more about how students adapt what they learn in the classroom to their non-academic lives. According to the entries in the student journals, students most frequently referred to information directly related to their everyday lives in non-academic contexts, usually information about food or addressing current events in the news. It was clear how writing to learn, and outside-of-class writing assignments impacted the transfer of knowledge. The findings of this investigation support utilization of news articles, writing prompts including narrative training to achieve this goal and our emphasis on the personal significance of each student's choice of topic.

13.8.5 Need for Scholarship of Teaching and Learning

A similar argument is that psychologists and educators should be enlisted to carry out these investigations since we lack the expertise necessary to carry out significant research in teaching and learning. Faculty members should conduct SoTL research to increase their effectiveness as teachers. The second component that flows from the first is the enhancement of student learning inside and outside the classroom. The advancement of education for individuals working in the field of phytopathology, if not the entire academic community, is a third reason why plant pathologists engage in SoTL research. Although education researchers and scholars have generated a large amount of data that is essential to our schools and could be used to improve our effectiveness as teachers, these individuals lack the time and motivation to adapt their data for learning to all of the fields of study in which it may be useful. Another benefit of including experts in phytopathology in SoTL research is the more prolonged exposure to phytopathology. Because we conducted research in our classes and then presented our findings at teaching seminars and workshops on campus or as presentations and posters at teaching-related professional meetings, many more people are now aware of the field of phytopathology. They are at least somewhat familiar that plant diseases affect their daily lives. We believe it is essential to do SoTL research in plant pathology, despite the many difficulties and perhaps unsatisfactory results.

13.9 A Glimpse into Experience of Technology Transfer in Extension

13.9.1 Technology Transfer in Extension

Technology transfer (TT) is an important step in the extension process, which involves sharing technical knowledge and innovation with the farming community (Koutsouris 2018).

13.9.1.1 Role of Internet

In its simplest form, the internet can be considered a vast network of computer networks. Text, graphics, photos, sound, and video are just a few of the several media types used to deliver information online. Staying updated is now possible in ways that were never before practicable. In 1997, the Van Buren County Extension Horticulture website included weekly updates on disease situations, including a fire blight pandemic. The Clarksville Horticultural Experiment Station regularly updates important data on the occurrence and prognosis of apple scab (Biggs and Grove 1998). In 2004, *Phakopsora pachyrhizi*, responsible for Asian soybean rust, probably invaded the country via the winds from Hurricane Ivan. With the introduction of the internet, extension advisories, publications, and diagnosis tools have continued to be available to people and other interested individuals.

With just a few clicks, growers may quickly access various educational resources, such as books, digital pictures, downloadable PowerPoint presentations with narration, and instructional video clips. Remote PowerPoint presentation access via personal computers is becoming more common in America (Vincelli 2010). Since some consumers find it difficult to distinguish between these disparities, recent efforts have been made to build websites that offer quality-controlled information about agricultural research. IPM Images is one example of an image database that is easy to use and access. One of the many searchable and user-friendly image databases accessible online is IPM Images. Fact sheets for several websites and a collection of diagnostic images for the NPDN are more examples (Vegetable MD Online). These websites are all consistently updated (Jacobson et al. 2008). Universities and some business interests have created web-based techniques that diagnose plant diseases without actual plant specimens and instead rely on digital pictures. The initial concept depends on the accurate GPS location of the seed tablet (Griepentrog et al. 2006). Real-time kinematic approaches (RTK) must be ordered from nearby vendors, and an extremely precise GPS signal is needed for position-based systems. The widespread use of smartphones and recent advancements in computer vision made feasible by deep learning have made smartphone-assisted disease detection conceivable. Smartphones, in particular, provide highly specialized methods to help with disease detection due to their computer power, high-resolution displays, and extensive built-in accessory sets, such as full HD cameras (Mohanty et al. 2016).

13.9.2 Alterations in the Role of Extension Specialist and Information Flow

Farmers can now find information from various sources, including seed distributors, fungicide manufacturers, advisors, fertilizers suppliers, information from other countries' extension programs, and peer-reviewed journal publications. Specialists should safeguard their standing as objective, science-based authorities by ensuring they uphold that standard in all they do if they want to ensure that extension remains applicable in this present era. Extension professionals have a unique position in that they may provide helpful research and recommendations that are typically viewed as objective and the most cutting-edge diagnostics, despite the fact that producers acquire information from several sources (Vincelli 2010). A rising number of programs in applied phytopathology use molecular tools to identify the pathogen. When *Phytophthora ramorum* causes rapid oak death, identification using nucleic acid-based methods is necessary to complete the diagnosis. Innovative technologies are helpful, but it's crucial to remember that to utilize them successfully, one must be aware of both their disadvantages and benefits. A recent review article on pathogen detection based on nucleic acid addressed these issues (Vincelli and Tisserat 2008).

13.9.3 Extension Programs in the USA

Extension programs have been implemented globally to enhance human capital by educating farmers on production techniques, the best input usage, and management techniques (Dinar et al. 2007). Most of the almost one million extension workers who provide daily advice to farmers worldwide are situated in low- and middle-income countries, particularly in Asia, where they make up 70% of the total (Bahal 2004). The West Africa Agricultural Productivity Program (WAAPP) is a significant extension initiative. The program was started in 2007 and operated in 13 countries in West Africa. It develops and disseminates climate-smart farming techniques to increase long-term agricultural productivity (Caiafa and Wrabel 2019). Plant pathology is involved in some of these areas, while it has little technical significance in many others. Despite numerous successes, critics claim that extension systems fall short due to low staff morale, financial strain, poor interactions with agricultural research, the improper use of extension officials for political ends, or the failure to maintain farmers' interest in training over the long term (Jones and Kondylis 2018).

13.9.4 Privatization of Extension Services in USA

It is crucial to remember that it is acceptable to question if a for-profit or a private company would be more appropriate to execute the services plant pathologists do as an extension in a capitalist society. However, extension specialists offer crucial services to community, including unbiased disease control advice, objectively applied research, and cutting-edge diagnostics for our forests, fields, and landscapes. Fortunately, at least among state and federal legislators, society appears to recognize this. There have been no significant proposals in the USA on privatizing extension services. This approach would damage both by severing ties between the extension and the land-grant university (Vincelli 2010).

13.10 Diagnostic Networks: A fruitful Tool for Plant Biosecurity

13.10.1 Significance of Plant Health

Most species, including humans, depend on plant systems for various ecological services. Systems for producing food and maintaining public health are founded on plant systems. It is exceptionally challenging to guarantee sustainable food security because of the complex networks involved in making food and transportation, reliance on availability and energy cost, and their vulnerability to political unrest (Anon 2008). Many medications are prescribed for cognitive and psychosocial issues and used to treat diseases obtained from plants. For the foreseeable future, plants will be essential in preventing rises in atmospheric carbon dioxide concentrations. If harnessing plant-based energy is a practical solution for reducing the negative consequences of climate change without jeopardizing food security,

healthy plant systems are required. People have prized plants for their aesthetic value throughout history and worldwide. The multibillion-dollar (US) sector of international trading in ornamental and landscape plants boosts many countries' local and global economies (Stack 2010).

13.10.2 Constraints to Sustainable Phytohealth

One of the many factors that can influence phytohealth is the obstacles created by biological weapons, bio crime, climate change, the increasingly globalized populace, and foreign trade (Stack 2008). Technology and research development will require financing to address these issues and safeguard the plant systems that sustain life and ecosystem preservation. One of the most pressing challenges is global plant and product commerce, combined with population growth and development. The relative significance of purposefully introducing plant diseases is difficult to assess, and there has been much debate on the subject (Young et al. 2008).

13.10.3 NPDN (The National Plant Diagnostic Network)

The Cooperative States Research, Education and Extension Service of the USA, in collaboration with the land-grant university system, established NPDN in 2002 to aid in protecting plants from the intentional, unintentional, and natural introduction of phytopathogens and pests (Stack et al. 2006). The primary goal of the NPDN's establishment was to identify disease outbreaks and the agents responsible for them quickly. Common diseases are most frequently identified based on the typical symptoms and objective evidence of diseases detected during diagnosis. The likelihood of misdiagnosis in the field depends on the expertise of the field specialist and the disease's local epidemiology. Getting laboratory confirmation of a diagnosis is essential for any disease with a significant risk of adverse consequences. As the diversity of the sample and the significance of the diagnosis increase, a diagnostic laboratory employs increasingly cutting-edge technologies. To identify regulated infections, extremely comprehensive molecular diagnostic approaches are typically needed (Stack 2010).

A thorough evaluation of the capacity and competency of each plant diagnostic laboratory was necessary due to the discrepancy in core competencies and diagnostic infrastructure among laboratories across the country. The idea behind triage is to differentiate between positives and negatives. The triage procedure, in concept, starts when the evaluation of the type of issue is made in the field at the epidemic site. This facilitates the quickest response possible by accelerating the diagnostic procedure.

Web-enabled microscopy and video conferencing are available at many NPDN laboratories. As a result, a diagnosis in a lab can send a picture of the material being examined under a microscope to a mini server, laying the groundwork for a functional telemedicine system. The URL to a webpage on the microscope server

is then communicated to an authority on that pathogen or host, for example, by email or phone.

A speedy and precise determination is one of NPDN's key objectives. In plant clinics, without widespread access to contemporary technologies, the physical and cultural characteristics of the pathogen are used for traditional diagnosis. This presents a few potential challenges for different infections. A diagnosis based on physical and cultural traits can take much time to improve the effectiveness and accuracy of diagnosing a plant disease. Through NPDN funding, grant funding, and assistance from host universities, the technologies necessary for current nucleic acid-based assays, like PCR and real-time PCR, were made available to NPDN laboratories. Thermocyclers, real-time thermocyclers, ELISA plate readers, gel documentation systems, and other variants of this equipment are available (Stack et al. 2006). For this laboratory network and screening system to be most effective, the results generated by each laboratory must be of specified quality to inspire confidence in the results and to permit the interpretation of shared information.

A national system for laboratory accreditation (LAS) is being created to fulfill this goal. Under this program, all NPDN labs would get the equipment and instruction required to meet the accreditation requirements for an NPDN diagnostic laboratory. Purdue University established a nationwide database for diagnoses from all NPDN labs. This data source presents insight into the regional prevalence rate of pathogens. Additionally provided is a database for epidemiological evaluations of the dynamics and trends of the disease. In the event of an intentional introduction, the data repository will give background data for forensic analysis (Fletcher 2008).

Using NPDN laboratories in a ring testing technique, USDA research facilities have enhanced the validation of novel diagnostic tools (Lamour et al. 2006). Providing the diagnosticians with extra training in the protocol before it was put into place as a standard operating procedure has improved the protocol validation. Additionally, the NPDN diagnosing labs have been requested to provide funding for studies comparing various diagnostic technologies in scenarios where an epidemic is present as part of research projects linked to disease outbreak response operations (Bullock et al. 2006).

13.10.4 Communications Infrastructure and Operations By NPDN

To enable information sharing amongst diagnosticians and regulatory agencies, NPDN created a secure communications infrastructure. The NPDN provides a web portal that acts as a public information resource and a gateway to each regional network's website. The communication system was designed to prevent unwanted physical or electronic access to network data and guarantee data accessibility and integrity against various human and environmental threats, such as vandalism and extreme weather conditions (Stack and Baldwin 2008).

13.10.5 Training and Education Program Developed By NPDN

An education and training program for first detectors was developed and implemented by NPDN. First responders are educated to recognize disease symptoms and indicators, as well as pathogen and insect damage that may have detrimental effects. NPDN training programs are presented in person, either in a classroom setting or in the field. There is also a functioning online system for first detector training that enables asynchronous program distribution.

To ensure the rapid and precise diagnosis of diseases and pests, Phyto diagnosticians must be instructed in using cutting-edge diagnostic technology and the most recent SOPs. The correct notification procedures, as well as the rules and laws governing the handling, storing, and transportation of specific agents, sample processing, and sample destruction, must all be understood by NPDN diagnosticians. The topics covered in the regional hands-on workshops provided by NPDN include insects (such as *Maconellicoccus hirsutus*), infections (such as Wheat Streak Mosaic Virus), diagnostic tools (such as PCR), and vectors (such as *Aceria tosichella*).

A method to help states prepare for an outbreak of a plant disease was developed and implemented by NPDN. Each exercise is based on a well-constructed issue that describes the protocols needed for a successful response to and resolution of an outbreak of insect pest or disease. Asian soybean rust, *Agrilus planipennis, Agrilus marcopoli,* including other epidemics, have been modeled in scenarios. A computer software program is used to manage each exercise, giving all the data required for detailed post-exercise evaluations.

13.11 An Overview of International Cooperation for Global Plant Biosecurity

Plant diseases seriously threaten world food security (Strange and Scott 2005). Because of the rising hazards associated with the widespread movement of plant products and plants across the world, international collaboration is essential for ensuring plant biosecurity (Stack and Fletcher 2007). The plant diagnostic facilities of business partners, bordering countries, and those situated along natural trade routes in North America should be connected to international networks (Main et al. 2001). The European Union created the Crop Biosecurity Program to introduce a research network focusing on harmful diseases of plants under the direction of the University of Torino (Gullino et al. 2008). This network aims to include more European and Mediterranean countries and secure communications technologies to enable coordinated diagnosis (Stack and Baldwin 2008). Regional labs have been introduced in Africa, the Caribbean, Central USA, and Southeast Asia through the NPDN paradigm. For regional plant diagnostic clinics, many nations have created and made diagnostic training programs available. In countries with limited resources, IPDN will substantially impact ongoing investment.

13.11.1 Plant Health Clinics and Plant Pathology Training

The agricultural industry plays a significant role in increasing nutrient-dense food, guaranteeing food security, and reducing poverty worldwide (Jones and Ejeta 2016). Despite the significance of agricultural crop production, insect and disease infestation generate problems along the value chain of crop production. Plant pests and diseases that are economically significant cause about 40% of agricultural output losses (Oerke 2006). Plant health difficulties can be treated by correctly diagnosing and identifying pests affecting agricultural plants (Ausher et al. 1996). Farmers can obtain advice on plant pest problems affecting crops through plant health clinics and management strategies for pest control (Alokit et al. 2014).

13.11.2 Training in Crop Protection at UCL

The main training objective has been to provide specialized training in plant protection without compromising the need for multidisciplinary skills and adapting to changes in agriculture and the job market ever since the Integrated Crop Protection opportunity was set up at our faculty in 1967 and throughout the numerous program revisions since then. The compulsory disciplinary training in crop protection includes lectures on organisms that affect plant development, such as viruses, bacteria, fungus, nematodes, arthropods, and weeds. Additionally, it discussed the interactions between pathogens and plants in epidemiology, case studies of harmful pathogen or pest interactions with plants in phytopathology and applied for entomology courses, and crop protection techniques in crop improvement, phyto pharmacy, biological, and integrative courses (Maraite et al. 2010). The phyto clinic course has been the basis of our plant protection curriculum at UCL since its inauguration in 1976. This course was created due to interactions with the plant clinic connected to the department of botany and phyto pathology at Purdue University in Indiana, USA, during the author's postdoctoral stay there in 1972. Applying the phyto clinic concept, as employed in an American land grant university, did not appear practical to the UCL scenario because developing a phyto clinic service was not UCL's top priority. The author defines a plant disease clinic as employing actual field samples of diseased plants, integrating scientific knowledge to understand, prevent, or treat the observed disease, and training plant doctors (Evans-Ruhl 1982).

The phyto clinic course begins in the fall with one or two outdoor sessions designed to teach students how to identify disease or pest injury symptoms, examine the distribution of the symptoms, gauge the prevalence and severity of the disease, and assess the likelihood of further development of disease. The following 10 to 15 weeks are spent instructing the students to diagnose illnesses in the lab more quickly and accurately. A Leica M3/M5 stereomicroscope, a Zeiss Axiomat light microscope with phase contrast and dark field, a box with tools and chemicals for slide preparations, and isolations of pathogenic fungi and bacteria are all present in the lab for the plant clinic course, which has 15 student workstations.

It is let out to identify particular cultures using PCR or electron microscopy in collaboration with a mycological lab. Small groups of students are also instructed on the practical use of ELISA tests and PCR testing for mycotoxin and fungus detection in addition to conventional procedures.

13.11.3 The Plant Clinic Service: A bridge that connects the University and Farmers

Under the auspices of CORDER, the plant clinic service has been created since 1985. Employees were initially compensated through a regional program to lower unemployment, supporting public interest initiatives. Due to service agreements with individuals and organizations, client fees, and occasionally a reimbursement from the Walloon Area for the social service provided, the budget has expanded to approximately €350,000 per annum. In compliance with CORDER, space may be rented from the UCL Lab of Phytopathology, major equipment may be shared, and the library and L3 Biosafety Laboratory may be used for work involving quarantine pathogens. As a result, the phyto clinic service acts as a conduit between farmers and the university.

13.11.4 The Linkage Between the Phyto Clinic Course and Phyto Clinic Service

The university's phyto clinic course and the non-profit plant clinic service work well together. On occasion, the course will incorporate additional analysis of intriguing samples sent to and evaluated by the service. Through their interactions with the service, the students learn about the purposes and specifications of a phyto clinic service. Both the course and the service share the paperwork for the phyto clinic. Around the time of their training's conclusion, which usually occurs in May, the most experienced students are allowed to participate in the analysis of specific samples delivered to the phyto clinic service. After the analysis, they provide the service bioengineer with their diagnostic and recommendations. These samples can be added to the herbarium for their Plant Clinic course. Many students make use of the plant clinic program. Furthermore, plans have been established for specialized instruction in the strategies employed by the plant clinic service (Maraite et al. 2010).

Specifically, in terms of phytopathology and the study of plant health, the plant clinic course helped increase one's awareness of adequate crop protection. The sharpest students may grow frustrated if there is a significant disparity in the group's pre-course training levels, even though they view the integration of knowledge through group discussion as a plus. Phytopathology training and plant clinics cannot be linked individually. Each university must choose the best strategy based on its resources, environment, and goals.

13.12 Technology Exchange Between China and Italy for Sustainable Crop and Environment Protection

Both highly developed and developing countries currently face new issues in agriculture. In developing countries like China, where 60% of the population still lives in rural areas, in poor conditions, and depends on agriculture as their primary source of earning, a shift toward sustainable agricultural systems which are more diverse (OECD 2005). A key component of sustainable agriculture is plant protection. Discussion is had regarding the results of an eight-year partnership between Italy and China in sustainable plant protection.

13.12.1 Significance of Agriculture and Agricultural Research in China

China is a major agriculture-based country. Considering the size and significance, China's agricultural economy is receiving much attention and becoming increasingly important to global trade. Nearly 15% of the GDP and more than 40% of jobs are accounted for by agriculture. Around 200 million farms, with an average land area per household of 0.65 hectares, are engaged in small-scale farming, dominating agricultural production structures (OECD-FAO 2008). China's agricultural performance would have a huge impact on the global food and agricultural markets and the fight against poverty worldwide (Dong et al. 2006). Plant protection has become a vital academic and professional area in China. Most agricultural colleges and universities offer this particular area of study, and most townships have technicians trained in plant preservation. Most agricultural science academies above the county level have established research facilities, crop protection, and quarantine.

From 1998 to 2006, the state allotted 2.58 billion yuan for constructing fundamental infrastructure for plant pest emergency control, monitoring, and early warning. The government budget provided 272 million yuan in 2006 to monitor yellow rust (*Puccinia striiformis*), snout moth larvae, and plagues of migratory locusts (*Locusta migratoria manilensis*) (*Crambus agitatellus*). Environmentally friendly pesticides increased from 3.1% to 18.1% in 2006, whereas highly toxic and lethal pesticides dropped from 21.8% to 11.8%. Through a combination of law and technical transfer, advancements in the safe use of pesticides are still being made. These efforts significantly enhanced plant protection methods, better working conditions, and improved protective capacity (Gullino et al. 2010).

13.12.2 Technology Exchange Between China and Italy in the Discipline of Sustainable Crop Protection

The Sino-Italian Cooperation Program for Environmental Protection was introduced in 2000 by the Italian Ministry for Environment, Land and Sea and the China State Environmental Protection Administration. Since then, this framework program has

carried out several projects in sustainable plant protection (Clini et al. 2008). Every project has as its primary goal the reduction of dependence of China on the use of chemicals, which poses serious concerns to both the environment and food safety. Finding alternatives to methyl bromide, a toxic fumigant used in the horticulture industry for pre-plant soil sterilization and banned by the Montreal Protocol because of its role in the depletion of the ozone layer, has cost much money since the inception of the Sino-Italian Cooperation Program (Gullino et al. 2003). Italy, which in the 1990s ranked second in the world for methyl bromide consumption and first in Europe for the production of horticulture products, developed significant expertise in the creation of workable and practical methyl bromide substitutes and made investments in the transfer of those technologies to other nations, particularly China (Gullino et al. 2003). Soil solarization, grafting onto resistant rootstocks, and the application of less harmful chemicals at reduced dosages via drip irrigation, tested on tomatoes and strawberries, were more well-liked by farmers despite providing levels of effectiveness comparable to methyl bromide. This is because they required less financial outlay and fewer adjustments to fit the traditional cultural practices (Dong et al. 2007).

Field tests under the Sino-Italian Cooperation Programme were conducted in Shouguang County, Shandong Province, east China, in 2006 and 2007. Three tomato rootstocks—Beaufort F1, Energy F1, and He-Man F1—were tested in one experiment to see how well they suppressed the root-knot nematode (*Meloidogyne incognita*) in a greenhouse environment. The results showed that the three rootstocks increased yields by 16–20% while reducing the occurrence of root knots by nearly 90% compared to a susceptible control (FA189 2008; Cao et al. 2008). Chemicals like flusilazole, carbendazim, metalaxyl, fenvalerate, and triadimefon are only a few examples that are no longer permitted in Europe and have been replaced with more natural and biological alternatives. The usage of pesticides on pears, for instance, was decreased by 29% and 69%, respectively, in comparison to traditional use, even though productivity was unaffected. In 2007, waxy maize pesticide use was reduced by 67% and 100%, while yields increased by 6% and 15%. Tomatoes and watermelons were cultivated in 2007 without the use of any chemical pesticides (Gullino et al. 2010)

13.12.3 Main Aim of Projects

The projects made an effort to adapt their objectives and methods of implementation to the particular locations they were focused on to adequately address those areas' unique social and economic needs, even though their primary purpose was to help China fulfill its obligations under the MEAMG. To discover, create, and carry out research and extension activities appropriately matched to local and global contexts, higher education institutions in China must develop the essential competencies. The inability to handle novel cropping systems and the lack of technical and scientific understanding have been two main obstacles to applying innovative techniques. Orienting scientific and technological expertise to meet market and industry

demands is a difficulty. Due to environmental and financial considerations, which served as a steady frame of reference for all projects, fruit and vegetable crops were selected as the target crops. Although grains are still China's main crop, their proportion of total crop yields and planted area both fell significantly from 1990 to 2003 as other crops, like fruits and vegetables, became more lucrative and the government relaxed most of the policy requirements that had previously pushed growers to grow grains (OECD 2005).

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