Sustainability in Plant and Crop Protection 13

Imran Ul Haq
Siddra Ijaz *Editors*

Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches

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Sustainability in Plant and Crop Protection

Volume 13

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Preface

Plant scientists, particularly pathologists, always are in a continuous struggle exploring new ways for plants protection, facing challenges such as adverse environmental and soil conditions and various kinds of diseases. The field of plant pathology is always challenging as it is of great importance for researchers to achieve the Nobel goal of food security and safety for an ever-increasing world population.

George N. Agrios mentioned in his book *Plant Pathology* that with a careful estimation, up to 42% losses in crops may be attributed to biotic stresses world over annually and these estimated losses are different from those caused by abiotic stresses. The situation is worse in developing countries where people face the challenge of food security, suffering from malnutrition and starvation. Plant scientists, therefore, always try to explore and develop more advanced, efficient, economic, and balanced ways to get maximum food yields. This may be achieved by protecting crops from diseases and insects, keeping the environment clean and healthy, and reducing any adverse effects on human and animal populations.

Moreover, advancement and evolution in biological science disciplines, such as microbiology, biotechnology, bioinformatics, and information and communication technology, offered new dimensions to plant pathology for the development of new disease management strategies. By keeping this perspective in view, we are making this attempt to keep plant scientists updated with latest developments in plant disease management strategies, aiming at the best integration of conventional and innovative methods.

We tried our best to collect and compile useful, practical, and recent information on plant disease management from diverse groups of authors and countries associated with well-reputed teaching and research organizations. We hope we reached the objective of updating and equipping readers with the most comprehensive and latest knowledge available today.

This book considers traditional and modern approaches for plant disease management. For a sustainable agriculture, methods based on sustainable management of phytopathogens are indeed an indispensable factor. In a nutshell, we tried to cover competitive areas of plant disease management, assembling best classical and modern strategies, most suitable for a sustainable agriculture.

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Chapter 1
History and Recent Trends in Plant Disease Control: An Overview

Imran Ul Haq and Siddra Ijaz

Abstract Plants are continuously exposed to certain biotic and a biotic stresses, causing serious crop losses every year. Prevailing situation is representing today a serious threat to global food security and safety. Any professional plant pathologist needs to have theoretical as well as practical knowledge and a clear understanding of plant diseases and of the factors involved, knowing how to discover effective control means. This chapter has been designed to provide the reader a brief overview regarding the concept of plant diseases, their diagnosis and the threats they pose to crop production and protection. Here we discuss and focus on basic principles including: plant disease management, conventional and advanced methods of controlling diseases and integration of various control measures, historical perspectives, disease management in the current era, future directions and challenges.

Keywords Plant pathology · Historical perspectives · Principles of plant disease control · Recent trends in plant pathology

1.1 Introduction

Plant pathology is the science concerned with a detailed study of plant diseases (caused by biotic and abiotic factors), mechanisms of inducing diseases in plants and efforts for their survival by overcoming diseases and achieving plants full genetic potential. The field of plant pathology is dynamic. It is worth studying all practical efforts needed to achieve the noble goal of providing safe and diverse food

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for our ever increasing global population. Plant diseases affect not only crop yields but also their quality, and reduce farmers resource-use efficiency. Plant health management strategies preventing crop losses (yield and quality) enhance production and significantly contribute towards food security and safety (Strange and Scott 2005). With an increasing world population and its food needs, the agricultural research in twentieth century remained focused on increasing crops productivity (Evans 1998; Smil 2000; Nellemann et al. 2009).

Plant pathogens mostly include microorganisms, such as fungi, bacteria, viruses as well as abiotic stresses such as adverse environments, lack or excess of nutrients, extreme temperature ranges, high moisture, dry condition, soil pH, pollution, light intensity and chemical injuries. Unwanted plants (weeds) are also a big growth constraint, causing heavy crop losses other than pathogens and insects. Damages caused by insects or animals to plants are not included in plant pathology.

Plant disease management strategies practiced as long as agriculture itself. Despite of all the scientific and technological advancements and their contributions in controlling diseases and significantly reduce the occurrence and severity of epidemics to date, plant protection is still a big challenge for agricultural scientists, and it is more complex than ever before (FAO 2011; Brown 2011). Plant pathogens (fungi, bacteria, viruses) not only interact one each other during the infection process, but also with other abiotic factors. Crop health management hence requires a multidisciplinary approach (Teng et al. 1984). To achieve the goal of sustainable plant disease management, some research areas need to be focused on. They are: host resistance development, pathogens avoidance or evasion, reduction of inoculum and remediation strategies, integration, set up of environmental conditions affecting pathogens reproduction and growth, evolution of new pathogenic races (He et al. 2016).

1.2 Plant Pathology: Food Safety and Security

Preventing the infestation and contamination of food from disease-causing microorganisms is important and represents a major concern for food safety. Food security is defined as the nutritious, healthy and uninterrupted food supply to all people around the world, for a healthy life style. Undernourished people suffering from food security issue in 2010 were 925 million worldwide, a highly unacceptable number (FAO 2010; Clapp 2014). By causing various diseases, plant pathogens affect crop plants ranging from merely mild symptoms to calamities, which may turn out to be famine or may intensify current shortage of food for million people. Plant pathogens have much variability in their population and can easily overcome resistance, thus ruining the epic work of plant breeders. Hence, conventional plant breeding equipped with latest plant disease management technologies, GMOs and marker-aided selection, have a crucial role to play in food security, powered by sufficient resources (Strange and Scott 2005). Oerke (2006) explained that food safety and security issues due to plant stresses, especially plant diseases, are severe in countries with less resources, which become critical when postharvest losses are included. Hundred of billion USD are wasted due to plant diseases every year, in terms of money. If global harvest and postharvest losses are combined these exceed 16–28% of global production, with higher percentages in developing countries lacking facilities and infrastructure to control spreading of plant pathogens (Agrios 2005). Recently, *Puccinia graminis tritici*, one of the most severe pathogen of wheat causing black stem rust, has damaged wheat crops in low income areas of Africa, Middle East and Asia, causing serious issues of food security (Flood 2010). Similarly, another example of trans-boundary impact of plant pathogens is *Fusarium xylarioides,* which has caused loss of almost 1 billion USD to coffee producers in central and Eastern Africa (Flood 2009).

1.3 Brief History of Plant Pathology

In the early stages of nomadism when leaves, fruits, seeds, and meat were the main source for survival, plant diseases were present and named as mildews, blights and blasts (Russell 2005). With the advancement in living style and a shift from nomadism to domestication, individual families began to grow crops, mainly cereals (wheat, barley, oat and rye), legumes (chickpeas, lentils and fava beans) and fruit trees (figs, apples, olives, peaches and citrus).Among other fruit crops grapes there were squash and melons and other plants also cultivated for survival of people and their animals as well. Around 470 BC the Greek philosopher Democritus mentioned the ways to control mildews, blasts and blights in his writings (Agrios 2005). Plant diseases were commonly considered at that time as divine punishments. Therefore, in fourth century BC, when Romans faced heavy crop losses by rust diseases, a deity called “Robigus”. They began to worship and offer sacrifices with the belief that this deity would prevent the dreaded rusts and other diseases (Littlefield 1981). Democritus suggested that sprinkling of olive grounds were helpful in controlling plant blights. Homer, described around 1000 BC the therapeutic properties of sulfur against plant diseases. However, most ancient reports dealt with pseudo-beliefs. Very little information was written since then anywhere for almost 2000 years.

In 1200 AD it was observed that mistletoe parasitizes the host plant and makes it sick. The host plant could be saved by the removal of infected plant parts. In the mid of sixteenth century, it was noted by French farmers that wheat rust infections were always more frequent and growing near barberry plants. Farmers believed that these plants played a key role as source of primary inoculum which on later stage infected the cultivated wheat fields. They hence requested the Government to pass out a legislation aimed at eradication of wild bushes of barberry to protect wheat (Agrios 2005). Meanwhile, it was observed that a species or variety was more resistant than others. Probably, in late 1600s southern England farmers used sodium chloride solution (brine) as seed treatment to control wheat bunt. Later on in mid-1700s the brine solution was substituted with copper sulfate which significantly improved the bunt disease control efficacy (Russell 2005). In 1670, Thoullier found that ergotism was caused by consuming contaminated grains and claimed that it did spread from person to person. In mid 1700s tree canker(s) were cured by excisions of the infected part(s) and grafting wax was used to cover the cut area (Agrios 2005).

In 1755, the French researcher Tillet proved through experimentation that smut infected plants could be increased by dusting the smut spores on wheat kernels before planting. Infection rates could be reduced by the application of copper sulfate to the wheat kernels before sowing (Tillet 1755). In 1761, copper sulfate was used to control wheat bunt (Schulthess 1761).

In the early 1800s, mildew of fruit trees was controlled by the application of either lime sulfur or aqueous sulfur solutions. In 1807, the French researcher Prevost properly concluded that wheat smut could be controlled with copper sulfate that could inhibit the germination of smutted spores (Prevost 1807). Late blight epidemic throughout Europe (particularly in Ireland) required scientists' efforts in controlling the diseases. In 1846, John Lindley observed the effectiveness of copper on potato plants but his report did not get popularity. By 1854, in England, powdery mildew infection on leaves and grapes was controlled by applying a mixture of lime and sulfur to the infected parts. The same practice was adopted by French vineyard industry for management of the powdery mildew of grapes, which caused heavy losses of up to 80% to their vine production industry (Spencer 1978; Kenrick 1833). In 1860s, another disease attacked the French vine industry. It was noticed that the Phylloxera aphid *Daktulosphaira vitifoliae* Fitch 1856 (syn. *Phylloxera vastatrix* Planchon, 1868), probably introduced with vines imported from the USA as powdery mildew resistant, was associated with this disease. However, grapevines in North America were resistant against these aphids so they were imported and used as rootstocks for the grafting of European vines. Some of these grafted vines showed excellent degree of resistance against the aphids.

In 1870s the German scientist Kuhn studied control measure strategies, to specifically cope with seed borne infections. In 1878, European grapevines were attacked by downy mildew. The attack was so severe that within 5 years of its appearance it spread throughout French and adjacent vine producing countries. Scientists were trying hard to cope with downy mildew and for this purpose they applied different substances to the soil or even dusted the vines, but nothing worked significantly. In October 1882, the French botanist Pierre Alexis Millardet was strolling through a powdery mildew affected grape vine orchard. He was surprised to see that vines alongside road were still green and healthy. By close observation of leaves he found that the vines were treated with some kind of chemical. Later on, by investigating from the orchard manager he understood that it was a common practice to treat the vines with a poisonous mix of chemicals (copper sulphate and lime) to protect them from passers. In 1885, he find out the best combination (8-8-100) to control downy mildew of grapes which is known today as Bordeaux mixture (Millardet 1885). The discovery of the Bordeaux mixture started the chemical control of plant diseases. The mixture was an excellent fungicides as well as bactericide. It was successfully used over a century to control several diseases (leaf spots, late blight of potato, leaf blights and downy mildews) throughout the world. This discovery improved the way of thinking that plant diseases could be successfully controlled by using chemicals (Agrios 2005).

In 1913, the concept of seed treatments with organic mercury compounds flourished which dominated until the 1960s when mercury was banned due to its high levels of toxicity. In 1928 Alexander Fleming, with the discovery of penicillin, came up with a different idea of controlling plant diseases. In 1934, discovery of the dithiocarbamate fungicide thiram led to the development of ferbem, zineb and maneb along with the development of many other protective fungicides. In 1965, carboxin was discovered followed by other systemic fungicides (Russell 2005). In 1950s, streptomycin was used as first antibiotic against bacterial diseases. Soon after, cycloheximide provided some promising results for management of fungal pathogens. In 1967, plant diseases caused by mollicutes as well as by fastidious bacteria were effectively controlled by tetracycline. In 1954 and 1963, a few bacterial and fungal strains were observed that developed resistance against a bactericide and fungicide.

The appearance of pathogenic race(s)/strains resistant to specific chemicals entirely changed the strategies of plant disease management. Strategies such as use of fungicides in combinations, alteration of compounds and application of systemic compound(s) at earlier stages followed by broad spectrum compound(s) on later stages of the diseases were extensively suggested (McManus et al. 2002). By 1980s, most of pesticides (85–90%) related to public concerns were banned either by US government, EU authorities or manufacturers. This initiative enforced the scientists' communities to re-examine and improve alternative disease control measures that were used by ancient times, which now represent the basis of integrated disease management (IDM, see Sect. 1.6).

In the early twentieth century it was reported that some microorganisms have the capability to harbor or suppress soil borne diseases caused by soil borne pathogen(s). Furthermore, the discovery of penicillin enforced the researchers to find out quite similar non-pathogenic microorganisms that could be able to antagonize or inhibit the pathogenic one(s). In 1963, the first biocontrol was obtained against root and butt rot of pines caused by *Heterobasidion annosum*, controlled by inoculation of *Phleviopsis gigantean* (a non-pathogenic fungus) to the stumps of freshly cut pines. In 1972, the crown gall bacterium of stone fruit was controlled by pre-inoculating seeds and root transplants with a related but non-pathogenic bacterium. Tobacco mosaic virus was controlled in tomato fields by pre-inoculation of seedlings with non-pathogenic strains obtained by artificial virus mutation. Control of citrus tristeza was obtained through cross protection. In late 1980s, control of viral diseases was obtained through genetic engineering techniques. Another recent development in plant disease control includes the systemic acquired resistance by creating necrotic lesions when pathogenic microorganisms are applied to the plants. In 1960s, plant defense activators were synthesized and market tested in 1996, with reasonable success (Agrios 2005).

1.4 Basic Principles of Plant Disease Management

Plant pathologist must have all kinds of information regarding the host plant, the pathogen attacking that particular host, the data about disease occurrence and history, agronomy, environmental conditions, cost of production etc., while planning and implementing the most appropriate disease management strategies.

Principles of plant disease management may be summarized as follows:

1. ***Exclusion***: this is probably the first defense line in IDM aimed at prevention of introduction and dispersal of inoculum into the area where it was not present before. ***Regulatory control methods***, usually adopted with the objective to exclude the inoculum from host plant, or from a certain area. These measures are applied by means of ***Quarantine and Inspections***, based on a number of regulatory practices. It is the responsibility of the national or state regulatory agencies to ensure the prevention of introduction and dispersal of the pathogens between and within the country or states, by implementing the quarantine laws (Fegan et al. 2004; Nutter and Madden 2005). Quarantine usually imposes complete restriction on import of any agricultural product for specific pathogenic threats, or may impose partial sanctions depending on the results of the material inspection. The import restrictions under the quarantine regulation might be imposed on all countries, provinces or regions (Fry 1982; Palti 1981; Sill 1982). Mayths and Baker (1980) suggested principles to make the quarantine regulations more effective. According them (i) the organisms suspected to cause damage to the crops should be restricted only through these regulations and (ii) these regulations should not affect the trade but only restrict the organisms capable of causing diseases. They should (iii) also be operated under the amended or improved law, according to the situation prevailing at the time.
2. ***Avoidance** or **Evasion***. Sometimes farmers select sites for crops cultivation usually based on a low risk of disease occurrence, due to certain factors such as unfavorable climatic conditions for disease development, absence of vectors, etc. This is a form of exclusion based on time. In time, avoidance of pathogen inoculum (by selecting proper planting date, site, seed or propagation material as well as agronomic practices and plant protection measures) is one of the effective strategy for controlling plant diseases. The philosophy involved in this strategy is to avoid the critical time period (favorable environmental and growth conditions) during which that pathogen may get established, causing infection and thus inducing crop losses. (Nutter and Guan 2001; Savary et al. 2006).
3. ***Eradication*** involves cultural (horticultural, agronomic practices), physical as well as biological and chemical methods aimed at reducing the initial inoculum of a certain pathogen from its host plant, plant part or from certain geographical area (site/field). Among the cultural practices, physical eradication of infected host, removal of infected plant parts, burial or burning of debris, eradication of alternate hosts and crop rotation are the methods most applied for eradication. Other practices such as soil solarization and mulching are more recent approaches, which are now widely practiced in different parts of the USA, Australia, and

many other countries, adding to sterilization, hot air treatment, hot water treatment, sun drying, Light wavelengths and refrigeration are physical methods used for inoculum reduction. Use of antagonists, trap crops, suppressive soil, cross protection are the biological approaches to reduce the pathogen population (Palti 1981; Coelho et al. 1999; Du Toit and Hernandez-Perez 2005).

Sanitation practices aim at reducing or eliminating a pathogen population from a diseased field, through disinfection of warehouses, tools and equipment that also may contribute in reducing the initial inoculum, using chemicals (Fry 1982; Lipps 1985; Palti 1981; Sharvell 1979).

4. ***Resistance*** is the ability of the host plant to defend itself against biotic or abiotic stresses. Some of plant pathologists keep host resistance under the "direct protection", one of the principles of plant disease management. However, the complex and scientifically interesting mechanism of host resistance to pathogens and the effects the disease development rates should be recognized as separate principles of plant disease management. The mechanisms of host resistance towards diseases may involve one or more mechanisms reducing the inoculum and the rate of infection and disease progress (Van der Plank 1963; Zadoks Schein 1979). There are two major types of resistance: (1) Resistance opposing the establishment of infection by reducing the amount of initial inoculum. It is also named vertical resistance, complete resistance, race-specific or monogenic resistance, and (2) other type of resistance opposing infection and disease severity on plants, called horizontal resistance, quantitative resistance, or partial resistance. Cultivation of resistant varieties is an easy to adopt, eco-friendly, safer and effective way to control plant diseases. Resistance breaks down, however, is a big challenge as new virulent races of pathogens evolve. Once a resistant variety is developed its useful "life span" may be enhanced by adopting proper agronomic, cultural, as well as plant protection measures.
5. ***Direct protection*** using chemicals for plant disease management is a significant component of disease management. Chemicals (fungicides) have been used to protect crop from pathogens since the 1940s. Since then, the application of fungicides contributed significantly in controlling plant diseases (Leadbeater and Gisi 2015). Replacement of older non-systemic fungicides with systemic fungicides (more effective and specific) was the major development in the field of chemical management in the '60s. For example among the systemic fungicides the triazole group gained a 24% business share of the total fungicide market (Hewitt 1998). On other hand, some non-systemic fungicides also had a significant business volume in developing countries, especially due to a lower cost. Development of new classes of fungicides posed significant effects on disease management. However, the resistance of pathogens to many of the newly developed products is still a big challenge for plant pathologists. Use of fungicides is more effective and efficient when combined with other control methods in an IDM program (De Waard et al. 1993). Actually, low toxicity, low residues in edible parts, and ecofriendly availability of agrochemicals to meet international health standards is a public demand (Knight et al. 1997). Since the development of first fungicidal formulations to date, a large number (likely several hundred)

formulations have been developed and made available commercially, worldwide. These fungicide formulations are applied in different ways, depending on the nature of targeted pathogens such as dusts and wetable powders, liquids, granular, and fumigants.

Several attempts have been made so far to improve the efficacy of fungicide formulations, by means of additional chemicals. Emulsifiers contribute in the formation of soluble suspensions. Foam suppressors increase the contact of the spray suspension maximally, on the plant surface. Penetrants enhance absorption capability of systemic formulation. Stickers enhance chemical persistence on plant surface. Surfactants reduce surface tension, and also enhance the penetration of the chemical to the plant subsurface. Wetting and dispersing agents improve particle suspension during application. UV filters enhance formulation photolytic stability on the plant surface (Agrios 2005).

The chemical industry branch for plant disease management started in nineteenth century with the discovery of simple inorganic copper and sulphur products. It is increasing its list of chemicals with complex, novel groups and various mode of action, effective against groups of pathogens (Hewitt 2000; Leadbeater and Gisi 2015). Thiram and captan are early broad spectrum, contact organic fungicides whereas streptomycin discovered in 1955 is still an effective and most common (when permitted) antibiotic, with gentamycin allowed in Latin America and oxolinic acid in Israel (Shtienberg et al. 2001; McManus et al. 2002).

Since 1930, new fungicides are providing a promising role to restrict and limit pathogens boundaries and for effective management of economically important diseases. A wide range of formulations and products for fungi and bacterial diseases are offered today on the market, whereas the nematicide industry showed minimum growth. This was most probably due to less awareness as indicated by few early reports (Hague and Gowen 1987). In the second half of the nineteenth century, carbon di-sulphide was discovered, a pioneer effective nematicide, followed by chloropicrin used successfully in early nineteenth century in England against nematodes and other soil pathogens (Schacht 1859; Kuhn 1881; Mathews 1919).

Fungicides target various known and unknown mechanisms of actions essential for fungal growth and development. Aniline-pyrimidines and streptomycin disturb protein synthesis and enzymatic activity; benzimidazole carbamates inhibit microtubule formation; hydroxyl-pyrimidine group fungicides attack metabolic processes involving nucleotides; chlorothalonil blocks glutathione conversion into its various forms (Hollomon and Chamberlain 1981; Chen et al. 2001; Halling et al. 2002; Gupta et al. 2004; Carr et al. 2005; Mueller et al. 2008; Yang et al. 2011; Koo et al. 2009).

Fungicides also have various mode of applications through fumigation, flooding, injector, dusters or as foliar, etc. according to the target pathogen. Least effective fungicides are continuously being replaced by chemicals with active redistribution and translocation ability, having novel, new chemistry and complex action sites such as mancozeb, propineb, captan etc. (Maude 1996; Klittich 2008; Ivic 2010; Milenkovski et al. 2010). For research and development of an effective new fungicide with distinct characteristics, huge amounts of funding and time is required, for its long term survival in market and safety to humans (Mcdougall 2010; Leadbeater and Gisi 2015). Pesticide rules and regulations are becoming strict with passage of time due to human and environmental health concerns, not hindering agricultural development, with USA, Brazil and the EU as leading examples (Pelaez et al. 2013).

Awareness regarding resistance against fungicides is important as it can waste resources utilized on products development and marketing. Various fungus and fungicide factors are responsible for resistance development (Brent and Hollomon 1995). Risk of resistance development vary in various groups of fungicides with strobilurin and benzimidazole at high risk, starting 2 years after the product introduction on the market (Brent and Hollomon 2007).

1.5 Biological Control

The exploitation of the antagonistic potential of microorganisms to control plant pathogens has gained popularity in recent years. Biological control is considered as the best alternative method to reduce the use of pesticides. It is a general consideration that biological control keeps environment healthy, eco-friendly, self-sustainable and has long lasting impacts. Several microorganism antagonistic to plant pathogens are available in the market, globally. The commercial formulations of these antagonists are named "microbial pesticides" or "biopesticides". Their possible modes of action includes parasitism, competition, antibiosis, induced resistance and inactivation of pathogen enzymes (Agrios 2005).

1.6 Integrated Plant Disease Management

In order to attain the target of a sustainable disease control in an economic and sustainable way, establishment and implementation an IDM system is the most appropriate option. While designing an IDM plan it must be kept in consideration that the system must include almost all possible control methods. Usually, an IDM system is designed with the objective of controlling all diseases of a single crop. However, it may also target a specific disease (major threat to a crop or occurring in epidemic form) e.g., potato late blight.

The major aims and components of an IDM program are: (i) eliminating or reducing the initial inoculum, (ii) keeping its effectiveness in time, (iii) enhancing the host resistance, (iv) slowing down the infection process and (v) the pathogen secondary cycles (Agrios 2005).

1.7 Recent Advances in Plant Disease Management

Modern plant pathology has been greatly accelerated with the aid of molecular tools and advancements in plant disease control strategies. Since the last few decades, molecular plant pathology has been proved very helpful by introducing several new ways and providing the better opportunities for disease diagnosis and control. In this regard, biotechnology and genetic engineering played a key role. Molecular techniques such as DNA-based identification of plant pathogen(s), rapid sequencing, quantitative real time PCR (qPCR), diagnostic assays, biomarkers, and whole genome sequencing greatly improved the way of pathogen(s) detection, disease diagnosis and management.

Field ready serological assays are very helpful in the decision making process and in pre-screening against targeted diseases. Biomarkers such as volatile chemicals are in practice for the detection of pest outbreaks. Biosensors coupled with information technology networks can provide real-time surveillance or monitoring of emerging problems caused by pests and diseases (Lucas 2011). Molecular tools such as microsatellites, remote sensing, image analysis, global information system, geo-statistics, and geographic information systems are very helpful in the monitoring and surveillance of plant diseases spread and risk assessment.

Development of different disease forecasting models and computer simulation-based methods for surveillance of plant disease epidemics are very important for their mapping (Agrios 2005). The adoption of the E-Phyto (electronic phytosanitary certificate) provides a great deal in safe trade of plants and plant-based products, by introducing innovative technologies. In recent years the potential of nanotechnology in plant disease management has been greatly put in practice. Surface response resonance and other nano-based sensors are very helpful in the detection of pesticides residues, seed borne mycoflora, seed certification and quarantine. Nano-based pesticides significantly reduced environment and health-associated risks.

Automatic purification of nucleic acids and specific proteins from pathogen(s) increased the possibilities of early diagnosis. The knowledge for identification of genetic basis, signaling molecules, network and pathways that control plant defense could be very helpful for the development of a new generation of genetic modulators. Additionally, it will provide more opportunities for the development of genetically modified organisms that could respond well to biotic stresses (Lucas 2011).

Plants have intrinsic networks to respond to phytopathogens upon their intrusion. They use a vast array of proteome and metabolome resources for their defense. Plant biologists are continuously struggling to explore the world of these biomolecules involved in plant-pathogen interaction and disease development. The resistance-associated genes have been tracking to be identified since a long epoch and are being used in the development of transgenic resistant plants. These genes are being used in the perspective of introducing resistance, triggering the endogenous resistance mechanism of plants by their overexpression against phytopathogens (Rommens and Kishore 2000).

However, advancements in the field of molecular biology facilitated to exploration of the molecular basis of plant pathogen interactions. Plant innate responses could be engineered to get durable resistance including systemic acquired resistance and hypersensitive response (Strittmatter et al. 1995). It could be fascinating to hypothesize that the introduction of resistance gene(s) would mediate incompatible reactions with annexing phytopathogens and lead to hypersensitive response ensuing localized cell death.

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Chapter 2
Plant Genetics and Physiology in Disease Prognosis

Ganesan Vadamalai, Lih Ling Kong, and Yasir Iftikhar

Abstract The dynamics of plant physiology and protein expression may largely contribute in disease diagnosis. Biochemical changes and secondary metabolites cross talk while pathogen and plant interact through cellular defense mechanisms. Plants genetics in relation to resistance levels vs pathogens helps in categorizing varieties and also the pathogen, on the basis of symptoms development. Although symptomology is the basic criterion for identification of plant diseases, other serological, biochemical and molecular assays are highly sensitive and useful for correct diagnosis of plant diseases. Advances in plant physiology and genetics, under varying spatio-temporal scales, are used for the detection and management of diseases. Thus, biochemical characterization of diseased plants opens new trends in disease diagnosis to formulate management strategies. In this chapter we focused on the comparison between genetics and physiology of diseased and healthy plants. Moreover, effect of biochemical changes due to certain pathogens on host plants are also discussed as concerns detection. The use of proteome in disease diagnosis is also described. Genetics of resistance and susceptible varieties vs diseases was highlighted for disease diagnosis. As different plant pathogens such as fungi, bacteria, nematodes, viruses and virus-like pathogens have different expression profiles during disease progression, physiology and genetics of diseased plants appear as useful tools for diagnosis.

Keywords Disease prognosis · Plant physiology · Plant genetics · Induced resistance · Biochemical detection

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2.1 Introduction

Any deviation from normal functioning in physiology, morphology and genetics of plant is referred as a disease. Therefore, certain biochemical changes and their expression also differentiate the diseased plants from healthy ones. Although symptomology is the first, basic criterion for the identification of plant diseases, other serological, biochemical and molecular assays are highly sensitive and useful for the correct and real-time diagnosis of plant diseases. Therefore, advances in the physiology and genetics of plant disease diagnosis are being used for the detection and management of diseases.

Plant pathogens are detected and characterized using different basis to manage the diseases in plants. Observation of symptomology and microscopic morphology are conventional methods for detection. Host-pathogen interactions are involved in physiological and genetic alterations present in diseased plants. Pathogens influence the physiological pathways and expression of many genes. Biochemical, serological and molecular assays have been recognized as recent trends in diagnosis. Biochemistry of diseased plants offers quick and reliable detection methods. Similarly, amplification of gene expression through different immunological and nucleic acid based assays are fast approaches that offer advantages over conventional methods.

Biochemical and molecular techniques are very useful against obligate fungal pathogens which are slow growing on the medium such as powdery mildew, downy mildew, and rusts etc. During a pathogen attack on the there are two types of host resistance/susceptibility reactions, such as compatibility and in-compatibility. The in-compatible reaction is involved in the hypersensitive response and production of some metabolites which help the plant to combat the invading pest or pathogens. In a compatible reaction the host is susceptible and conducive to the pathogen growth. Biochemical and molecular assays are important for a reliable disease diagnosis. Physiological characterization always involves many biochemical assays, instead of a single test as applied in morphology based diagnosis.

2.2 Recent Trends in Biochemical and Molecular Detection of Plant Pathogens

Plant pathogens are detectable on the basis of their specific biochemical activities in the host, and are detected by biochemical and molecular techniques such as electrophoresis, Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), dot blot hybridization, DNA finger printing etc. Study of DNA is fundamental for molecular detection of any pathogen. Analyzing DNA of plant and pathogens may show the alteration induced in physiological traits of diseased tissue samples. Electrophoresis is helpful in separating the complex mixture of DNA into fragments of different sizes. Polyacrylamide or agarose medium are required for electrophoresis, depending on the DNA or protein profile analysis. Protein profiling is performed through the reaction of different restriction enzymes, and is separated through electrophoresis. The electrophoresis analysis has been found very effective to detect and characterize the disease caused by many pathogens, over the last decades. A derivative of electrophoresis named “Isozyme electrophoresis” is being used effectively to differentiate species and strains. Grouping within the species of true fungi and oomycetes can be achieved through iso-enzyme electrophoresis. Using this assay different species of *Phytophthora* (*P. cinnamomi P. cambivora* and *P. cactorum*) can be separated. A phylogenetic tree may be constructed using iso-enzyme analysis. For example, virulence and non-virulence in black leg of canola was investigated to this analytical approach. *Fusarium* species distributed around the world have also been differentiated through cellulose-acetate electrophoresis (Oudemans and Coffey 1991a, b).

Viroids are the smallest pathogens infecting plants. Though they are short length RNAs, they have genetic variations. Different viroids have been detected and characterized through polyacrylamide gel electrophoresis (PAGE) and hybridization. Potato spindle tuber viroid, isolates of *Coconut cadang-cadang viroid* (CCCvd), citrus viroids, isolates of citrus bent leaf viroids have been characterized through PAGE (Morris and Wright 1975; Randles 1985; Hodgson et al. 1998; Barbosa et al. 2005; Cao et al. 2009; Khoo et al. 2017). Similarly, viruses and their isolated may be separated through PAGE and blotting (Narayanasamy and Doraiswamy 2003). Citrus viruses and different isolates of citrus tristeza virus have also been characterized through electrophoresis (Narayanasamy 2008).

2.3 Physiology of Diseased Plants

Plants pathogens exist in different strains, isolates, pathovar, races and biotypes, depending on their genetics. Gene for gene hypothesis (Flor 1946) opened different perspectives in genetics to be studied. Using conventional methods for strain identification is a time taking procedure. Pathogens are able to cause diseases in plants when one or more pathogenicity genes are present. With the passage of time different races, strains and biotypes evolved due to factors such as environment, competition for the host and point mutation in genetic makeup. Screening to find out the resistance source against different pathogens is useful to formulate management strategies. Genetic variability can also be determined through analyses of differential hosts.

Cultivars having different resistance genes have been selected and used to identify pathogen races physiologically different. This is the best way to define races in host plants, to avoid confusion. There are many examples of interaction between hosts and pathogens. Potato (*Solanum* tuberosum) interacts with *Phytophthora* infestans when the conditions are favorable for both of them. R1 resistance genes are present in the varieties of potato against virulent strain of *P. infestans* race 1. However, some fungal strains are complex and virulent to varieties having more than one resistance genes. For example, *P. infestans* virulent races named 1, 2 and 3 show virulence to specific resistance genes present in the potato that are indicated as R1, R2 and R3 resistance genes. Some cultivars having no resistance genes are susceptible to all *P. infestans* races that cause infection and lead to the minimal quality and yield losses. Another example is the interaction of the fungus *Cladosporium fulvum* and tomato *Lycopersicon esculentum* (Higgins and de Wit 1985). This interaction shows the specificity of the resistance genes in tomato against the virulent genes present in the pathogen genome. In case of plant pathogenic bacteria, the races are defined on the basis of avirulence genes, against the resistance genes present in the host plant. *Pseudomonas syringae* race 6 has the 6 avirulence genes acting against the resistance genes present in soybean (*Glycine max*) plants (Staskawicz et al. 1984). The races of *Xanthomonas campestris* pv. *malvacearum* are similarly defined (Gabriel et al. 1986).

There are different levels of resistance in different plant parts, according to age, and in genetically identical plants as well as in different parts and tissues (Innes 1974; Jones and Hayes 1971; Mares 1979). Rates of resistance increase or decrease in different plant parts as in case of stem and roots resistance, that increases in first 2 weeks as compared to fruits and leaves where resistance level decreases with age (Hunter 1978; Hunter et al. 1978; Jones and Hayes 1971; Nilsen et al. 1979; Wheeler 1977). Leaves show more resistance at developing stage, usually when flowering on plants occurs. These changes are referred to as ontogenic changes in plants, against the pathogen attack (Bell 1980). When a bacterial pathogen enters into host plant the host defense mechanism is activated, showing enhanced levels of resistance to infection by causing necrosis or local lesions. Such type of resistance is referred to as induced resistance, which is sometime induced by different chemicals in plants (Matta 1971). Various reviews are available in the literature on the resistance mechanisms active in plants after bacteria and nematodes attacks (Kuc and Hammerschmidt 1978; O'Brien and Fisher 1978).

Induced resistance develops similarly in different plant species with minor variations due to the effects of host species and inducing agents involved. A typical example of induced resistance is that developed against the bacterial pathogen *Pseudomonas lachrymans* (Caruso and Kuc 1979). The initial density of the inducing pathogen is very important for the extent and duration of the subsequent induced resistance. First appearance of systemic induced resistance in true leaves after inoculation occurs after 72–96 h. It reaches the highest level within in 7 days and continues for 4–7 weeks. Another inoculation made on a higher leaf gradually expands and persists for longer period until fruiting, and is called booster inoculation. After 96 h of inoculation the leaves removed show few lesions and induce resistance stands for a longer time, even after removal from plant (Jenns and Kuc 1979).

Bacterial multiplication is restricted by the high level of general resistance in the hosts, without involving necrosis of plants, and race-specific resistance occurs when high level of necrosis is present. Multiplication of virulent strains occurs exponentially until necrosis occurs and then populations drop. Population decline indicates the accumulation of antibiotics and other reactive chemical compounds in intercellular spaces between the lesions formed by the pathogen (Webster and Sequeira