Modern Approaches in Plant Pathology

Modern Approaches in Plant Pathology

Dr. Satish Sharma
Dr. S. K. Arsia
Dr Arvinder Kaur
Poorvasandhya R
Sunita Dhaka

–EPH–
Elite Publishing House

First Edition 2023

ISBN : 978-93-58990-37-9

Published by:

Elite Publishing House
A-10/28, Sector - 18, Rohini, New Delhi - 110089
Tele Info: 9289051518, 9289051519
Email: ephinternational@gmail.com, ephpublishers@gmail.com
Website: www.elitepublishing.in

Acknowledgment

Contents

Preface

1 Management of Evolving Pathogen for Durable Resistance

Amaresh Hadimani[1*], Chetana Veerendra Kalammanavar[2], Virupakshi Hiremata[3] and Vani N.U[4]

[1]Ph.D. Scholar Department of Plant Pathology, Kittur Rani Chennamma, College of Horticulture, University of Horticultural sciences, Bagalkot, Karnataka - 587104
[2]Project Assistant, ICAR-AICRP on Fruits, KRC College of Horticulture, Arabhavi, University of Horticultural sciences, Bagalkot, Karnataka - 587104
[3]Senior Research Fellow Division of Vegetable Crops ICAR- Indian Institute of Horticultural Research, Bengaluru-560089
[4]Senior Research Fellow ICAR-AICRP on Fruits, ICAR- Indian Institute of Horticultural Research, Bengaluru-560089

Abstract

No resistance endures permanently in an evolutionary sense. A resistance's level of durability may be thought of as a quantitative feature; resistances might be very durable or completely non-lasting (ephemeral or fleeting). According to experts, fungi and bacteria with a limited host range exhibit ephemeral resistance. A hypersensitive response (HR), substantial gene inheritance, and several resistance genes many of which often appear in multiple allelic series and/or complicated lociare its defining traits. These resistance genes (alleles) interact gene for gene with the pathogen's avirulence genes (alleles) to produce an unfavourable response. By causing a loss mutation in the associated avirulence allele, the pathogen counteracts the action of the resistance gene. The pathogenicity is reinstated and the incompatible response is no longer induced. Without losing fitness, the pathogens may tolerate the loss of several avirulences. Durable resistance to specialised bacteria and fungi is often quantitative and dependent on the cumulative effects of many genes, resulting in a different kind of resistance than the hypersensitive response. In most commercial

cultivars, this quantitative resistance to practically all diseases is found at low to good levels. Durable monogenic resistance also exists, and it's often of the non-HR kind. Resistance to bacteria and fungi with broad host ranges is often quantitative and long-lasting. Even when they are based on HR resistances that are monogenic, race-specific, viral resistances are often very resilient. The degree of specialisation does not seem to be related to how long a resistance lasts.

Go back young man and gather up your weary and defeated genes of the past, take your currently successful genes, find some new ones if you can, and build yourself a genetic pyramid.(Nelson, 1978, p. 376)

Keywords: Evolving pathogens; Durable resistance; Host and Pathogen

Introduction

It is commonly recognised that dangerous parasites have the ability to harm and infect their hosts. Thus, virulence is the primary characteristic of infections, and for a very long time, one of the main objectives of pathology, particularly plant pathology, has been to comprehend the development of virulence. Important phenomena like the emergence and reemergence of pathogens, host switching, host range expansion, and overcoming host resistance may be influenced by the evolution of virulence and may jeopardise the efficacy of control measures for infectious diseases affecting humans, domestic animals, and plants. Furthermore, diseases have a significant influence in determining the dynamics and composition of ecosystems (Bull, 1994; Ebert and Hamilton, 1996; Frank, 1996; Read, 1994), which may be modified through virulence evolution. Harris *etal.*, 2020, reported that among the top ten unanswered question in molecular plant microbe interaction, "**How do pathogens evolve novel virulence activities**?" is the second last question asked at about 1400 accomplices at 2019 International Congress on Molecular Plant-Microbe Interactions meeting held at Glasgow.

Evolving Pathogen

Epidemics of plant diseases brought on by the invasion of exotic fungal infections are a well-known occurrence. An explosive disease outbreak may be caused by the host's low level of resistance and the pathogen's too aggressive behavior (which reflects the absence of past coevolution). Events surrounding the introduction provide the disease a window of opportunity for evolution. A plant disease is often exposed to regular selection restrictions in the area where it is endemic, favoring the preservation of a population structure that is comparatively stable, although variable, over time. It will often be exposed to novel or episodic selection when brought into a new habitat. This reflects abrupt exposure to new biotic and abiotic variables, such as a new host population, new vectors, new competitors, or a different temperature. These factors

give rise to the possibility of quick evolution (Brasier,1995).

Both qualitative and quantitative resistance to disease have historically been identified in plants. Major genes, which provide phenotypically total or partial resistance to the virus, are genetically responsible for controlling qualitative resistance. It is based on gene-for-gene interactions, where the protein produced by the pathogen's avirulence gene is specifically 'recognized' by the plant's equivalent resistance gene (Flor, 1971). Following this identification, the plant has a hypersensitive reaction that confines the pathogen to the main infection site. In so-called "boom-and-bust" cycles, the development of the pathogen's virulence often follows the deployment of key genes providing qualitative resistance (Johnson, 1961; Parlevliet, 1989). According to Poland *etal*. (2009) quantitative resistance is often regulated by a number of genetic variables (minor genes) in the plant that provide partial resistance to the pathogen and slow the spread of epidemics over time. Additionally, there is mounting evidence that pathogen genotypes that may overcome quantitative resistance are being chosen (Burdon *etal*., 2014).

The reason why the bacteria evolve quickly is because they reproduce at a fast rate. Mutations of bacteria produce new strains. Some bacteria might become resistant to certain antibiotics, such as penicillin, and cannot be destroyed by the antibiotic. The evolution of the bacteria is an example of natural selection.

One of the most relevant consequences of novel virulence activities is the ability to infect new hosts, which ultimately leads to the emergence of new diseases (McLeish *etal*., 2017).

We have some knowledge about the mechanisms for overcoming the defences and exploiting host resources by pathogens, but we still do not know the key mechanisms that lead to the adaptation to a new host and, thus, determine pathogen host range (Morris *etal*., 2019).

Pathogen host range may vary from one or very few to several hundred different plant species (specialists versus generalists pathogens), although this may be difficult to determine, depending on the definitions of host and adaptation to host (Morris *etal*., 2019). Determinants of host range may be both intrinsic (availability of virulence factors that allow the interaction with different hosts) and extrinsic (e.g., exposure of plants to microorganisms and environmental conditions favorable for infection) (McLeish *etal*. 2017) Extrinsic determinants include ecological factors, such as host population structure and diversity, epidemiological, such as vector availability and dynamics, or even stochastic events (Brown and Tellier 2011). Host range evolution has mostly been studied by focusing on the intrinsic, genetic factors, but studies on the role of extrinsic factors are starting to gain importance (McLeish *etal*. 2017a).

Adaptation of pathogens to new hosts may lead to host range expansions and host jumps, the latter when the ability to infect a new host leads to genetic differentiation of pathogen populations on different hosts and, finally, pathogen speciation (Thines 2019). It is broadly observed that pathogens tend to infect plants that are closely related, rendering the phylogenetic distance between plant taxa as an important predictor of the risk of a new host acquisition (Gilbert *etal*. 2012; Schulze-Lefert and Panstruga 2011). However, there are also many examples of related plant pathogens that are able to infect distant hosts, so other factors such as host geographical, ecological, or physiological distance may play a role (McLeish *etal*. 2017; Morris and Moury 2019; Thines 2019). This is the case of new host acquisitions by indigenous pathogens when a host is introduced in a new area, such as the case of Cocoa swollen shoot virus, which was a pathogen of the native forest tree *Cola chlamydantha* before.

Management Stratergies Against Plant Pathogens

Durable disease resistance

Is defined as resistance that has remained effective while a cu1tivar possessing it has been widely cultivated in an environment favoring the disease. This characteristic of resistance is recognised retrospectively, as are all other characteristics of interactions between hosts and pathogens (Johnson, R.1983).

Disease resistance genes are a scarce resource, and it is expensive to introduce them into new kinds. It is consequently very difficult to maintain their effectiveness over time (i.e., their durability). According to Johnson (1981), a plant's ability to withstand illness is lasting if the cultivar that has it is extensively grown. The length of time it takes for the selection of pathogen genotypes to overcome the resistance and make the resistance gene useless is a measure of how durable a disease resistance gene is. The pathogen genotype frequency that must be attained for the resistance to be deemed broken down relies on the socioeconomic situation and is obviously arbitrary. The biology of the targeted pathogen (e.g., ploidy, reproductive mode, mutation rate) and other intrinsic aspects of the pathosystem, such as the mechanism and genetics of the molecular interaction, are all important factors that affect durability (Brown, 2015). However, it also relies on outside variables (such as agronomic practises and climatic circumstances) that have an impact on the fitness of the targeted species (Brown, 2015). Only by modifying these external conditions can the resistance genes' efficiency be maintained after they have been inserted into plant kinds.

Combining disease management methods with disease resistance genes might possibly increase the longevity of disease resistance genes since doing so maximises the effectiveness of all available "weapons" Applying fungicides that target the same pathogen, for example, may boost the longevity of a resistance gene that targets a

pathogenic fungus. Sadly, relatively few models or empirical investigations have taken into account such mixtures of diverse selection forces to postpone the establishment of virulence. Iacono et al. (2013) shown theoretically that the addition of a source of demographic stochasticity, such as occasional fungicide treatments, increases the longevity of a resistant cultivar.

To manage the diamond back moth, Plutella xylostella, Onstad et al. (2013) created a theoretical model incorporating insecticides, parasitoids, and transgenic insecticidal crops. They demonstrated how different insect control strategies worked together to boost each other's endurance. To fully understand the potential and constraints of such pairings, further research is urgently required. The use of pesticides may be one of the restrictions.

Plants employ a great variety of defense mechanisms to cope with the multitude of organisms that try to exploit plants.

The defense mechanisms can be classified in

1. Avoiding,
2. Resistance and
3. Tolerance mechanisms

Avoiding is especially used against animal parasites as these have sensorial abilities. Tolerance, enduring the parasite with relatively little damage, does not seem to play a significant role with pathogens. In breeding against pathogens it is resistance,that is used almost exclusively. Resistance mechanisms, reducing the growth and/or development rate of the pathogen, are nearly always of a biochemical nature. Soon after resistance to pathogens was introduced it became apparent that pathogen populations could adapt to such resistances; the resistance "broke down".

Strategies for the deployment of disease resistance genes

One important external aspect that significantly affects the longevity of disease resistance genes is their deployment in the field. When there are several sources of resistance genes present at once, planning opportunities based on the distribution of these genes throughout time and place are made possible. These tactics may be divided into four groups:

(1) The insertion of multiple resistance genes into a single plant (pyramiding)

(2) The use of multiple resistance genes in various plants within or between fields (multiline and variety mixtures)

(3) The periodic switching of multiple resistance genes at the same site (rotation); and

(4) The use of each resistance gene until the resistance conferred by it is broken down and replaced by a new resistance gene (sequential release).

The key global issues must be taken into consideration when talking about lasting resistance. According to predictions made by Alexandratos and Bruinsma (2012), Tilman *etal*. (2011) between the years of 2005 and 2050, there will be an increase in demand for agricultural production of between 60 and 110 per cent, and there may be even more of a rise in demand for forest products. According to studies by Garrett *etal*. (2006), mean variations in temperature and precipitation may have a positive, negative, or neutral effect on some disorders. The predicted rise in climate variability, which might increase the number of important illnesses and pests in a particular region and the annual swings in their prevalence, may be of more concern. The most effective control strategy often is host plant resistance for social, economic, and environmental reasons.

Non-durable resistance

In an evolutionary sense, every kind of resistance is temporary. However, there are significant variations in how easily parasites may get beyond a resistance. The longevity of a resistance varies widely in agriculture as well. As in the case of the Phylloxera aphid resistance of grape (*Vitisvinifera* L.) rootstocks, resistance may already have been neutralised in the latter phases of the breeding programme (at zero years), and may still be effective after more than 130 years and widespread exposure (Niks and Butler, 1993). And there are obvious distinctions among the non-durable kinds of resistance as well. Numerous main gene resistance and all QR based on one to several genes proved to be durable forms of resistance adopted by breeders that have not yet completely broken down (Parlevliet and Alemayehu 1997).On the other hand, certain patterns are discernible. There are several forms of resistance that have been quite elusive, with effective times varying from months to years. This obviously short-lived resistance is usually always under the control of a significant gene that also regulates the hypersensitive response. These genes function in a gene-for-gene manner with the pathogen's avirulence genes. Most of the pathogens are specialised fungi or bacteria, including cereal rusts, cereal powdery mildew, downy mildews of lettuce and lima beans, flax rust of flax, rusts of maize, late blight of potatoes, blast of rice, bean rust of common beans (*Phaseolusvulgaris* L.), anthracnose of common beans, and bacterial blight of rice (Parlevliet, 1993).

Important breeding techniques for conferring wide breadth and long-lasting resistance to diseases that reduce rice output include marker-assisted selection and gene pyramiding. Bacterial leaf blight (BLB), which is brought on by the pathogen *Xanthomonas oryzae pv. oryzae*, is one such disease that significantly reduces rice yield.

Numerous molecular marker approaches have previously been developed because molecular markers are crucial for both marker-assisted selection and gene pyramiding. Currently, DNA-based markers, sometimes referred to as molecular markers, are the most frequently employed ones. Based on the methods used to find them, the molecular markers are divided into two main types. These indicators are based on hybridization and polymerase chain reaction. The morphological (traditionally based) and bio-chemical (enzyme-based) markers are two more kinds of markers that are accessible. The most effective method for managing the BLB disease of rice is host plant/varietal resistance.

Table1: Number of years that the resistance to yellow rust in wheat (*Triticumaestivum*) cultivars and to powdery mildew in barley (*Hordeumvulgare*) cultivars remained effective in The Netherlands. (Anonymous, 1955-1994)

Wheat cultivar	Year	Barley cultivar	Year
Tadorna	1	Ramona	3
Clement	1	Mazurka	4
Heines V II	4	Sultan	5
Felix	15	Belfor	8
Arminda	18	Minerva	20

Gene pyramiding with the use of markers offers the potential to speed up breeding operations and ensure that the host plant's acquired resistance will last. This research explores the use, economic significance, constraints, and potential of marker-assisted selection and gene pyramiding for rice BLB disease resistance. The creation of BLB disease-resistant rice cultivars is particularly promising using marker-assisted selection.

Deployment Strategies

One gene at a time

There are circumstances when the use of a single main gene may be justified, despite neither breeders nor pathologists often advocating it. Due to reduced population proportions, resistance is more likely to persist in circumstances where the pathogen is less prevalent (Johnson, 1993). Therefore, a single resistance gene may be sufficient for a very long time in circumstances where a disease occurs at a moderate or low intensity, releasing resources to breed for durability to more serious/frequent illnesses. A second example would be the spread of new, particularly aggressive disease populations or pathogen species. Even while it is preferred to save resistant genes for use in a deployment strategy of some kind, it can be essential to employ single genes to maintain an industry's viability until longer-lasting tactics can be devised.

Gene rotation

Gene rotation entails the deployment of an efficient resistance gene, replacement with a different gene after the emergence of a virulent race, and reuse of the original resistance in the future following a sufficient reduction in the frequency of the related race (Crill, 1977). Although gene rotation strategies have been used to combat rice blast and rice tungro disease (Manwan et al., 1985), it is difficult to assess the effectiveness of these efforts (Mundt, 1994). Gene rotation is often accompanied with two significant challenges. The first is the very challenging logistical need of precisely tracking virulence, having sufficient supplies of replacement cultivar seed on hand, and securing permission from all farmers to switch cultivars at once. More importantly, once the associated resistance develops, virulences may not return to their initial frequencies the gene is removed.

Gene pyramiding

There is broad agreement that combining genes for resistance (gene pyramids) is a useful approach for increasing durability, with many known successes. The practice of mixing two or more genes from various parents to create better lines and variations is known as gene pyramiding. Pyramiding is the process of merging or stacking numerous genes, which causes many genes to be expressed at once in different species. Molecular identifiers make it easier to choose the best plants to use moving forward. In agricultural research, the word "gene pyramiding" refers to a breeding strategy for disease prevention and increased crop output. Gene pyramiding is a novel branch of plant breeding that has emerged as a result of the advancement of molecular genetics and related technologies like marker-assisted selection. There are two distinct components to the gene pyramiding technique. A pedigree, which is the first section, seeks to combine all the target genes into a single genotype known as the root genotype. The fixation phase, which is the second stage, tries to make the target genes homozygous, creating the optimum genotype from a single genotype. By lowering the number of generations that breeders must examine to make sure they have the appropriate gene combination, molecular marker genotyping helps speed up the gene pyramiding process. A crucial method for improving germplasm is known as Gene pyramiding (Rajpurohit *etal*., 2003). The most effective method for managing the BLB disease of rice is host plant/varietal resistance. Gene pyramiding with the use of markers offers the potential to speed up breeding operations and ensure that the host plant's acquired resistance will last. This research explores the use, economic significance, constraints, and potential of marker-assisted selection and gene pyramiding for rice BLB disease resistance. The creation of BLB disease-resistant rice cultivars is particularly promising using marker-assisted selection.

Conclusion

Combining disease management methods with disease resistance genes might possibly increase the longevity of disease resistance genes. Applying fungicides that target the same pathogen, for example, may boost the longevity of a resistance gene that targets a pathogenic fungus. Sadly, relatively few models or empirical investigations have taken into account such mixtures of diverse selection forces to postpone the establishment of virulence's. The addition of a source of demographic stochasticity, such as occasional fungicide treatments, increases the longevity of a resistant cultivar. To manage the diamond back moth, created a theoretical model incorporating insecticides, parasitoids, and transgenic insecticidal crops. They demonstrated how different insect control strategies worked together to boost each other's endurance. To fully understand the potential and constraints of such pairings, further research is urgently required. The use of pesticides may be one of the restrictions. In spite of their advantages in terms of plant protection, their effects on human health and the environment may sometimes be too severe for stakeholders to tolerate. More generally, it is recommended that agricultural practices (such as preventative measures, disease resistance genes, pesticides, biological control, or the use of beneficial organisms) designed to control a specific pathogen be combined to increase their individual and overall durability(REX Consortium 2016). Financial, organizational, human health, and environmental considerations that should be considered on a case-by-case basis in practice limit such combinations. Finally, it would be a good moment to revise Nelson's recommendation from 1978, which is as follows: Go back, young guy, and collect your most effective genes, chemicals, natural enemies, and practices. Then, create a very resilient approach, being sure to make it both economically and environmentally sustainable.

References

Alemayehu, F., & Parlevliet, J. E. (1997). Variation between and within Ethiopian barley landraces. *Euphytica*, *94*, 183-189.

Alexandratos, N., & Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision.

Brasier, C. M., & Buck, K. W. (2001). Rapid evolutionary changes in a globally invading fungal pathogen (Dutch elm disease). *Biological Invasions*, *3*, 223-233.

Broers, L. H. M., & Parlevliet, J. E. (1989). Environmental stability of partial resistance in spring wheat to wheat leaf rust. *Euphytica*, *44*, 241-245.

Brown, J. K., & Tellier, A. (2011). Plant-parasite coevolution: bridging the gap between genetics and ecology. *Annual review of phytopathology*, *49*, 345-367.

Brown, T. A. (2015). *Confirmatory factor analysis for applied research*. Guilford publications.

Bull, J. J. (1994). Virulence. *Evolution*, *48*(5), 1423-1437.

Burdon, J. J., Barrett, L. G., Rebetzke, G., & Thrall, P. H. (2014). Guiding deployment of resistance in cereals using evolutionary principles. *Evolutionary Applications*, *7*(6), 609-624.

Crill, P. (1977). An assessment of stabilizing selection in crop variety development. *Annual Review of Phytopathology*, *15*(1), 185-202.

Ebert, D., & Hamilton, W. D. (1996). Sex against virulence: the coevolution of parasitic diseases. *Trends in Ecology & Evolution*, *11*(2), 79-82.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual review of phytopathology*, *9*(1), 275-296.

Frank, S. A. (1996). Models of parasite virulence. *The Quarterly review of biology*, *71*(1), 37-78.

Garrett, K. A., Dendy, S. P., Frank, E. E., Rouse, M. N., & Travers, S. E. (2006). Climate change effects on plant disease: genomes to ecosystems. *Annu. Rev. Phytopathol.*, *44*, 489-509.

Gouilleux, F., Wakao, H., Mundt, M., & Groner, B. (1994). Prolactin induces phosphorylation of Tyr694 of Stat5 (MGF), a prerequisite for DNA binding and induction of transcription. *The EMBO journal*, *13*(18), 4361-4369.

Haddad, W. M., Chellaboina, V., & Rajpurohit, T. (2003, June). Dissipativity theory for nonnegative and compartmental dynamical systems with time delay. In *Proceedings of the 2003 American Control Conference, 2003.* (Vol. 1, pp. 857-862). IEEE.

Harris, J. M., Balint-Kurti, P., Bede, J. C., Day, B., Gold, S., Goss, E. M., ... & Alvarez, M. E. (2020). What are the top 10 unanswered questions in molecular plant-microbe interactions?. *Molecular Plant-Microbe Interactions*, *33*(12), 1354-1365.

Johnson, M. K., Hashtroudi, S., & Lindsay, D. S. (1993). Source monitoring. *Psychological bulletin*, *114*(1), 3.

Johnson, T. W., & Sparrow, F. K. (1961). Fungi in oceans and estuaries. *Fungi in oceans and estuaries.*

Levine, D. M., Ek, W. E., Zhang, R., Liu, X., Onstad, L., Sather, C., & Vaughan, T. L. (2013). A genome-wide association study identifies new susceptibility loci for esophageal adenocarcinoma and Barrett's esophagus. *Nature genetics*, *45*(12), 1487-1493.

Manwan, I., Sama, S., & Rizvi, S. A. (1985). Use of varietal rotation in the management of tungro disease in Indonesia. *Indonesian Agricultural Research & Development Journal*, *7*(3-4), 43-48.

McLeish, M., Sacristán, S., Fraile, A., & García-Arenal, F. (2017). Scale dependencies and generalism in host use shape virus prevalence. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1869), 20172066.

Morris, C. E., Lamichhane, J. R., Nikolić, I., Stanković, S., & Moury, B. (2019). The overlapping continuum of host range among strains in the Pseudomonas syringae complex. *Phytopathology Research*, *1*, 1-16.

Nelson, R. (1978). Genetics of horizontal resistance to plant diseases. Annu. Rev. Phytopathol. 16, 359–378

Niks, R. E., & Butler, G. M. (1993). Evaluation of morphology of infection structures in distinguishing between different Allium rust fungi. *Netherlands Journal of Plant Pathology*, *99*, 139-149.

Parlevliet, J. E. (1993). What is durable resistance, a general outline. In *Durability of disease resistance* (pp. 23-39). Dordrecht: Springer Netherlands.

Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C., & Nelson, R. J. (2009). Shades of gray: the world of quantitative disease resistance. *Trends in plant science*, *14*(1), 21-29.

Read, A. F. (1994). The evolution of virulence. *Trends in microbiology*, *2*(3), 73-76.

REX Consortium (2016) Combining Selective Pressures to Enhance the Durability of Disease Resistance Genes. Front. Plant Sci. 7:1916.

Rietveld, C. A., Medland, S. E., Derringer, J., Yang, J., Esko, T., Martin, N. W., & Preisig, M. (2013). GWAS of 126,559 individuals identifies genetic variants associated with educational attainment. *science*, 340(6139), 1467-1471.

Schulze-Lefert, P., & Panstruga, R. (2011). A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends in plant science*, *16*(3), 117-125

Thines, M. (2019). An evolutionary framework for host shifts–jumping ships for survival. *New Phytologist*, *224*(2), 605-617.

Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the national academy of sciences*, *108*(50), 20260-20264.

2 Integrated Approach in Plants Disease Management

Dr. S.K. Arsia[1], Dr. OP Bharti[2] and Dr. Satish Sharma[1]

[1]RVSKVV Gwalior, Dept. of plant Pathology, B.M. College of Agriculture Khandwa (M.P.)
[2]J.N.K.V.V.-Krishi Vigyan Kendra, Kolipura, Tappar Indore Road, Harda (M.P.)

Abstract

Plant diseases are caused by various biotic constraints that lead to significant crop yield loss over the globe. Plant diseases could be managed by combining several methods. The use of holistic combination of biological, cultural, physical and chemical control strategies under integrated disease management (IDM) is proved to be more effective and sustainable rather than using a single component strategy. Conventional cultural, Physical, mechanical and chemical methods are generally followed by most of the farmers for plant disease management as a single strategic manner in which chemical methods have immediate effect on the plant pathogens and hence most of the farmers are following though it is having the deleterious effects whereas biological control methods with disease resistance are having slow effect but management is permanent and having good impact on plant growth promotion. Nevertheless, the strategy of IDM use is gaining Importance, but in developing countries it often lacks the enabling environment for its successful implementation. As the scenario of farming for the last few decades clearly showed that adoption of IDM and participatory approaches helped farmers improve their overall crop management, including disease management, reducing costs and improving production efficiency. Integrating all these method to manage the plant disease is known as Integrated Plant Disease Management.

Keywords: Integrated, Disease management, IDM, Plant, and approach

Introduction

Integrated disease management (IDM) can be defined as a decision-based process in which multiple tactics and strategies of disease management are coordinated to optimize the management of pathogen on ecofriendly and economical feasible way. The IDM is an alternative approach of conventional use of physical, cultural, biological and chemical as single component strategy in which all the approaches are used in combination with the judicious and limited use of chemicals with the main objective to keep the disease incidence below economic threshold level. IDM may include site selection and preparation, utilizing resistant cultivars, altering planting practices, modifying the environment by drainage, irrigation, pruning, thinning, shading, and applying pesticides, wherever required. But in addition to these traditional measures, monitoring environmental factors (temperature, moisture, soil pH, nutrients, etc.), disease forecasting, and establishing economic thresholds are important to the management scheme (Khoury and Makkouk, 2010).

Crops are infected by many of the pathogens and cause diseases which lead to quantitative and qualitative crop yield loss. It is an estimate that 10-15% of low crop yields in developing countries due to disease attack, and losses may be higher if post harvest diseases are considered. It is known that more than 800 million people do not have enough food, and around 1.3 billion live on less than one dollar a day in the developing countries at present (FAO, 2004). It is a study that all production constraints including diseases for six major crops viz. wheat, rice, sorghum, chickpea, cassava, and cowpea in Asian and African farming systems losses caused due to diseases ranged from 3 to 14%, however yield losses was ranged 16 to 37% due to all biotic factors and yield losses to all crop production constraints ranged from 36 to 65% (Waddington *et al.*, 2010). In India, management of diseases in crop is most important because of the Indian foreign exchange and economy greatly depends on farming. The diseases management practices are one of the tool to strengthening of farm production which will promote the crop yield. The objectives of IDM practices are as follows-

- Management of wide range of pathogen simultaneously
- Reduction of chances of entry of pathogen
- Regular monitoring of the spread of the disease
- Elimination of any condition which influence the pathogenic growth and spread
- Integrated management of multiple diseases

It has been observed during last few decades, diseases in crops occurred in high intensity tremendously and great advancement also have been seen in disease

management practices. Diseases can be managed by several advance practices like cultural, physical, chemical, biological, breeding, biotechnological, tissue culture and molecular methods. It is also observed that ancient and modern researches against plant diseases with their combinations can made absolute plant disease management. Each and every method has some merits and demerits. Therefore use of suitable techniques and methods in combinations to manage disease below economic injury level which is known as "Integrated Disease Management (IDM)". The Principles of diseases management are the main stem of Integrated Disease Management. The principles behind in IDM approach are Exclusion, Avoidance, Protection, eradication, Host resistance and chemotherapy

Strategies and Tactics: The definitions of the **strategies** generally an overall plan for reaching a particular objective is called a **strategy**. Strategy is a path why which the object of disease management can be achieve or in other words steps involved in the sequence to get the perfect disease management of particular crop or particular disease in an area we can called as strategies. Strategies may involve many steps like supervision, monitoring Avoidance, chemical treatment, sowing times, seed treatments etc. but the important thing is the decision when and where these component can be applied. While the specific means for implementing a given strategy are called **tactics**. Any endeavor that requires a series of connected tasks for its completion also requires some kind of overall plan. Each individual task, no matter how skillfully executed or how successful its outcome, will not advance progress toward the final objective unless it has a coherent relationship with all of the other necessary tasks.

Principles of Integrated Disease Management

Studies on etiology, symptoms, pathogenesis and epidemiology of plant diseases are interesting and scientifically justified but it is very important that they are helpful in development of methods for successful management of plant disease. The practices of disease management vary from one disease to another depending upon the type of host and pathogen. Principles of plant disease control were first classified by Whetzel (1929) into exclusion, eradication, protection and immunization. Later in advancement and development of new methods of strategies of plant disease control two principles - avoidance and therapy were added (NAS, 1968).

Avoidance

The principle avoidance applied by planting at time when or in areas where, inoculums is absent or ineffective due to environmental conditions. The major aim is to enable the host to avoid contact with the pathogen or to ensure that the susceptible stage of the plant does not coincide with favorable conditions for the pathogen. The main practices under avoidance are choice of geographical area, selection of the field,

choice of sowing / planting time, selection of seed and planting material, disease resistant varieties and modified suitable agronomic or cultural practices. For example- The potato cultivation at high altitude is relatively free from viruses; as prevailing environmental conditions do not permit the buildup of vector populations. Similarly, early planting of potato or wheat, in indo Gangetic plains may escape late blight or stem rust damage respectively.

Exclusion

This principle Exclusion is preventing introduction of new disease causing agent or pathogen from invading in new or uninfected areas/region, farm, or planting material where inoculum absent and contact between pathogen and crop should also be avoid. It is nothing but prevention that inoculums should not be enter or established in a field where it does not in existence. Some of the means like Seed certification, inspection, eradication of inoculums, eradication of vectors, and quarantine measures are used to prevent the spread of pathogens.

Eradication

The termed as eradication is a principle to minimize and elimination or destroying inoculums from the source, either from a region or an individual plant where it is already established. One of the most extensive eradication operations carried out during 1927- 35 in the USA for management of citrus canker (*Xanthomonas axonopodis*) in which 4 million citrus trees were cut and burnt to eradicate the pathogen. The alternate and / or collateral hosts, crop rotations, field sanitations, heat treatment or chemical treatments of planting materials or soil fumigation etc. can be applied under this eradication practices.

Protection

In case of the principles of avoidance, exclusion and eradication cannot be applied to prevent the pathogen infection for many fast spreading infectious pathogens, brought by wind or irrigation from associated fields or any other distant place of survival. So that it can be achieved by using toxic barriers between the host surface and the pathogen. The application of chemical sprays, dusting, application nutrients, host management, vector management may be applied.

Resistance

Resistance to diseases is also a genetically controlled character under this principle we can utilizes cultivars that are resistant or tolerant to infection. The concept of vertical and horizontal resistance was suggested by Vander Plank in 1968. Disease resistance may also be defined as the capacity of the host to defend itself against pathological processes or the agents of those processes. All most all the case of diseases

resistance, infection or certain degree of establishment of pathogen takes place. Usually resistance can reduce the degree of establishment, rate of spore production and colonization. Resistance is the ability of a plant to reduce the growth and or the development of the parasite after contact has been initiated or established. It utilizes in-built mechanism to resist various activities of pathogen. The basic breeding techniques like selection, mutation and hybridization, use of biotechnological tools such as tissue culture, genetic correction and fusion are being used to develop resistant. Resistance in host can be described of two type, vertical and horizontal resistance.

Vertical resistance: it is a race specific, pathotype specific or simple resistance. It is generally determined by major genes or few specific genes and is characterized by pathotype specificity. In general it can manage the single disease

Horizontal resistance is generally controlled by polygene, many genes and is pathotype nonspecific, common resistance. It can slow down the reproductive rate of the pathogen so the disease spread slowly. It is also known as race nonspecific, partial or general resistance. In case of horizontal resistance, it can covers or control more than one disease. The horizontal resistance is the natural resistance where most of the pathogen occurs in low virulence.

Monogenic: It is controlled by specific single gene. This type possesses high resistance to a given strain or race of the pathogen but it is susceptible to other races.

Polygenic: It is controlled by many genes and is not so high but at the same time does not easily breakdown due to the evolution of new races. This is also referred to as durable resistance.

Therapy

Therapy is a curative procedure to protect plants that are already infected or is applied to individuals after infection. It is used on individual plants and cannot be used on a large scale. It can be achieved by treating the plant with some chemicals, antibiotics, and metabolites etc. that inactivate the pathogen. a principle of plant disease control, it provides an opportunity to cure or rejuvenate the diseased host plant by use of physical or chemical agents.

Chemotherapy is the use of chemicals to inactivate the pathogen, whereas heat is sometimes used to inactivate or inhibit virus development in infected plant tissues and heat may also kill the pathogen is known as T**hermotherapy**.

Components of integrated disease management

IDM is currently defined as: "a sustainable approach to managing diseases by combining biological, cultural, physical and chemical tools in a way that minimizes

economic, health and environmental risks". Accordingly, the major components of integrated disease management summarized here are: Quarantine and Regulatory measures, Cultural control, Physical and mechanical control, chemical control, biological control and Host resistance. Even though these components will be dealt with individually, it should be mentioned that often the different components are complementary to each other with strong interaction among them and the environment.

Quarantine and regulatory measures

Plant quarantine is defined as the legal enforcement measures aimed to prevent the spread of disease from the area where they have already gained entry and have established in new restricted areas, by the restriction on movement of planting materials. As a principle, quarantine is one of the most effective weapons against plant diseases. Quarantine is classified into three categories, namely Domestic, International and Embargo quarantine. Under the DIP Act, the Directorate of Plant Protection, Quarantine and storage has the responsibility to take the necessary steps and regulate the inter-state movement of plants and plant material in order to prevent the further spread of destructive insects and diseases that have already entered the country. Most of the states in India have plant quarantine laws to avoid entry of plant pests and diseases. There are many limitations to implementing domestic plant quarantine in India due to the vastness of the country and the unrestricted movement of plant material from one state to another. For implementation of quarantine regulation at the international level, proper check is maintained at the points of entry at airports and seaports. At present plant quarantine regulations differ with different countries for major agricultural commodities that are being exported out of India.

Cultural practices:

Cultural practices play an important role in plant disease prevention and management. The benefits of cultural control begin with the establishment of a growing environment that favors the crop over the pathogen. Reducing plant stress through environmental modification promotes good plant health and aids in reducing damage from some plant diseases.

- Deep ploughing of the field results in exposure of propagules to elevated temperatures and physical killing of the pathogen. This can be regarded as dry soil solarization.
- Flooding of the field somewhat resembles soil disinfestations. Long-term summer soil flooding, with or without paddy culture is found to be decreased populations of soil borne pathogens.

» Sanitation practices aimed at excluding, reducing, or eliminating pathogen populations are critical for management of infectious plant diseases. It is important to use only pathogen-free transplants.

» In order to reduce dispersal of soil borne pathogens between fields, stakes and farm equipment should be decontaminated before moving from one field to the next. Reduction of pathogen survival from one season to another may be achieved by crop rotation and destroying volunteer plants.

Physical and mechanical measures:

Mechanical and physical Practices can kill pathogens directly or make the environment unsuitable for it. The common methods are:

1. Collect and destroy the disease infected plant parts.
2. Soil sterilization at 50-60^{o}C for about 30 min kills the all soil borne pathogens.
3. Some seed borne diseases like loose smut of wheat (52^{o}C for 11 min), leaf scald (50^{o}C for 2-3h), red rot (54^{o}C for 8 h) and ratoon stunting of sugarcane (50^{o}C for 3h), black rot of crucifer (50 ^{o}C for 20-30 min) etc. can be treated by hot water treatment by immersing infected seeds in hot water at recommended temperature and time.
4. Hot air treatment is given to remove excess of moisture from plant organs and protect them from fungal and bacterial attack. Several virus infected dormant plants are treated by hot air treatment at a temperature ranging from 35-54^{o}C for 8 h.

Chemical control

Chemical control is widely used to maintain green leaf area and increase grain yield. Fungicide treatment has been found to be effective when the infection level is visually more than 5% of the leaf area (Cook *et al.*1999). The economical benefit should be achieved from proper timing of reduced fungicide doses, which give substantial increases in net yield and cost-effectiveness. Another strategy is the proper choice of fungicide; its dose and application time are important in achieving economic efficacy. Seed treatment is one aspect of crop management. It is an advanced and economic delivery system to protect the genetic potential of the seed against diseases from the moment of sowing and also partial replacement of the conventional foliar application. Seed treatments with fungicides have antagonistic activity against pathogenic fungi on seed-borne and root rot diseases and are capable of suppressing root rots as well as other plant diseases.

More recently, new classes of fungicides were developed with significant

impact on disease control. These include anilinopyrimidines, phenoxyquinolines, oxazolidinediones, spiroketalamines, phenylpyrroles, strobilurins and activators of systemic acquired resistance. However, the development of pathogen populations showing reduced sensitivity to many of the newly developed products posed a serious challenge that the traditional fungicides

Biological control

Success in using microorganisms against plant pathogens started with the control of crown gall with *Agrobacterium radiobacter* K84 (Kerr,1980), and that of seedling blights caused by *Pythium* and *Rhizoctonia* with *Trichoderma harizanum* (Harman and Bjorkman, 1998), *Gliocaladium virens* (Lumsden and Walter, 1995) and *Streptomyces griseus* (Cook *et al.*, 1996). However, the use of naturally occurring bio-control agents (antagonists) of plant pathogens can be traced back to many centuries through the traditional practice of crop rotations that primarily permit the reduction of pathogens' inoculum potential in the soil below injury level. Cross protection is also a one of the modern bio- control method for the disease management. In this method mild strain of virus or microorganisms is inoculated in host plant. These provide protection of host plants from those viruses and microorganisms which may cause much more severe damage e.*g.* papaya ring spot disease, citrus tristiza disease, *etc.* One of the limitations of using bio control agents is their inability to survive in certain field conditions. However, bio control agents have the ability to improve disease management when integrated with other management options.

Some of the benefits of an integrated approach are as follows:

- » Promotes sound structures and healthy plants
- » Promotes the sustainable bio based disease management alternatives.
- » Reduces the environmental risk associated with management by encouraging the adoption of more ecologically benign control tactics
- » Reduces the potential for air and ground water contamination
- » Protects the non-target species through reduced impact of plant disease management activities.
- » Reduces the need for pesticides and fungicides by using several management methods
- » Reduces or eliminates issues related to pesticide residue
- » Reduces or eliminates re-entry interval restrictions
- » Decreases workers, tenants and public exposure to chemicals

» Alleviates concern of the public about pest & pesticide related practices.

» Maintains or increases the cost-effectiveness of disease management programs

Conclusion

The disease occurs in plant is by biotic and abiotic means and causes a significant loss in agriculture system. The success and sustainability of IDM strategy, especially with resource poor farmers greatly depends on their involvement in helping to generate locally specific techniques and solutions suitable for their particular farming systems and integrating control components. The success and sustainability of IDM strategy, especially with resource poor farmers greatly depends on their involvement in helping generate locally specific techniques and solutions suitable for their particular farming systems and integrating control components that are ecologically sound and readily available to them. Integrated disease management is the practice of using a range of measures to prevent and manage diseases in crops. Hazard analysis is used to identify the potential for infection so that preventative or curative measures can be put in place to minimize the risk of disease infection and spread. During the cropping cycle, regular crop monitoring is used to decide if and what action is needed. Integrated Disease Management is one of the best solutions for mitigate the disease in vegetable crops through cultural, biological, mechanical and chemical methods.

References

Cook RJ, Hims MJ, Vaughan TB. Effects of fungicide spraytiming on winter wheat disease control. Plant Pathology, 1999, 48, 33-50.

Cook RJ, Bruckart WL, Coulson JR, Goettel MS, Humber RA. Safety of microorganisms intended for pestand plant disease control: a framework for scientific evaluation. Biological Control, 1996, **7,** 333-351.

FAO, 2004. FAOSTAT: FAO statistical data bases. Rome, Italy (http://faostat.fao.org).

Harman GE, Bjorkman T. Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and growth enhancement. In: Harmon G.E., Kubicek C.P.(eds). *Trichoderma and Gliocladium,* Enzymes, Biological Control and Commercial Applications, 1998; 2:229-265.

Khoury, W. El. and Makkouk, K. 2010. Integrated plant disease management in developing countries. *J Plant Pathol*, 92 (4, Supplement): S4.35-S4.42.

Kerr A. Biological control of crown gall through production of agrocin 84. *Plant Disease,*1980; 64:25-30.

Kakraliya SS, Abrol Sonali, Choskit D, and Pandit Devanshi. Integrated Disease Management in Agriculture. *www.justagriculture.in, Vol.1 Issue-3, November 2020.*

Kumar V, Ram RB, Meena ML, Shukla V and Singh C. Integrated disease management through organic farming in Vegetable crops in india: a sustainable tool. Asian Academic Research Journal of Multi-Disciplinary 2013, Volume-1, Issue-7.

Lumsden RD, Walter JF. Development of biocontrolfungus *Gliocladiumvirens*: risk assessment and approvalfor horticultural use. In: Hokkanen M.T., Lynch J.M. (eds).Biological Control: Benefits and Risks, Cambridge University Press, Cambridge, UK, 1995; 263-269.

Pandey AK, Sain SK, and Singh Pooja.A Perspective on Integrated Disease Management in Agriculture. *Bio Bulletin* (2016), Vol. 2(2): 13-29.

Park D. Antagonism the background to soil fungi. In: The Ecology of Soil Fungi Parkinson, D. and Waid J S. Liverpool, Liverpool University Press, 1960, 154-160.

Pauliz TZ. Low Input No-till Cereal Production in the Pacific Northwest of the U.S. The Challenges of Root Diseases. European Journal of Plant Pathology. 2006; 115:271-281.

Rasool K, Rasool F, Bhat N A, Mushtaq N, Wani TA, Alam S, Sushil Kumar, Saxena A, Bhat S, and Yousuf Shanaz. IDM: A new approach to disease management. Int. J. Chem. Sci. Volume 2; Issue 2; March 2018; Page No. 07-11

Thurston H.D., 1990. Plant disease management practices of traditional farmers. *Plant Disease* **74**: 96-101.

Thurston H.D., 1992. Sustainable Practices for Plant Disease Management in Traditional Farming Systems. Western Press, Boulder, CO, USA.

Tripathi L., Mwangi M., Abele S., Aritua V., Tushemereirwe W., Bandyopadhyay R., 2009. Xanthomonas wilt: A threat to banana production in East and Central Africa. *Plant Disease* **93**: 440-449.

waddington S.R., Li X., Dixon J.,Hyman G., de Vicente C., 2010. Getting the focus right: production constraints for six major food crops in Asian and African farming systems. *Food Security* **2**: 27-48.

Whetzel, H. H.1929. The terminology of phytopathology. hoc. 2nd Int. Congr. Plant Sci, Ithaca 2:1204-15

3 Plant Pathology and Crop Losses: Economic Impact and Management

Dharani Sureshkumar[1] and Priyadharshini Eswaran[2]

[1]Ph.D.Scholar, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu)
[2]Ph.D.Scholar, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu)

Abstract

The pathogens responsible for the diseases of crop plants cause a serious problem in terms of economy and also human health problems. These diseases are deadly that it could drastically reduce the yield under favourable conditions and also produces deadly chemicals which on intake can lead to death of humans. The economic losses due to diseases were estimated to be 12 to 14% and the post-harvest losses caused by pathogens were estimated to be 4.61 to 15.9%. Researches have been carried out since to control the diseases and increase the yield for the sake of economy and also for the well-being of people. In this chapter, the economic impact of the diseases and the management strategies developed so far have been discussed in detail.

Keywords: Economic impact, disease management, fungal disease, bacterial disease, viral diseases

Introduction

Plant diseases substantially minimise the yields of food crops, which has downstream repercussions for human health as well as loss of species variety and mitigation costs associated with control techniques. In many parts of the world, outbreaks of plant diseases are on upward trajectory and pose a threat to those who rely on food security. At the moment, a worldwide epidemic is endangering the health of millions of people worldwide. The agroecosystem favours the emergence of new, host-specific, "domesticated" crop pathogens that evolve more quickly and are more virulent than their "wild" ancestors. This is because agroecosystems have homogeneous genetic and

physical environments, which create a selective environment that is very different from that found in natural ecosystems. Compared to natural ecosystems, agro-ecosystems have higher planting densities and more genetically homogenous host populations, which facilitate disease transmission and boost pathogen virulence in the agro-ecosystem. By raising the overall number of mutations available in agro-ecosystems and decreasing the effects of genetic drift, these variables also increase the size of the pathogen effective population, resulting in more pathogen genetic diversity.

To help people out of poverty and improve health outcomes, a reliable, healthy food supply will be necessary. Climate change, transmission through international food trade networks, pathogen propagation, and the emergence of novel pathogen lineages are all contributing to the spread and aggravation of plant diseases, both endemic and recently developing (Ristanio *et al.*, 2021). Plant disease epidemics are also a major threat which dates back to the Great Irish famine of 1845 to 1852 in Ireland. Hence the cultivation and consumption of food crops, as well as the social and political stability of nations, are all severely impacted by plant diseases, which are a global problem.

In order to meet the growing demand for food's quality and quantity, plant protection in general and the ability of crops to defend against plant diseases in particular, are clearly important. Eradiction of the pathogen is not completely possible and hence management is the best possible way. To provide a consistent and ongoing supply of marketable produce for the expanding global population, disease management is of paramount importance. The increased use of chemicals in disease control has had a negative influence on environmental quality and led to an increase in the number of plants that are chemically resistant. The application of increasingly more biological control agents appears to be the only technology that has promise for managing diseases without upsetting the equilibrium of the harmful and beneficial composition of the environment and ecosystem (Kumar *et al.,* 2014). The new era of agronanotechnology has its roots in nanoscale science and nanotechnologies, which have the potential to transform food and agricultural systems.

Economic Impact Caused by Plant Pathogens

Plant pathogens impair agricultural production's productivity and quality. At the household, national, and international levels, they result in significant economic losses and reduce food security. It is challenging to compile and compare quantitative, standardised data on agricultural losses across crops, agroecosystems, and geographic regions. Disease injuries brought on by epidemics may result in crop loss (damage), which may then result in economic loss.

Crop diseases can coexist with more hosts in agro-ecosystems, increasing the possibility that multiple genotypes (strains) of the same pathogen will infect the same plant at the same time. Due to competition among strains for the same host resources, multiple infections also support the development of increased virulence. Due to competition among pathogen species for the same host resources, the increased host and pathogen density found in agro-ecosystems enhances the possibility of co-infection by diverse pathogen species, which also favours higher virulence. The possibility of horizontal gene transfer is also increased by co-infection with various disease species, as has been shown for several crop infections that have acquired genes altering virulence, sometimes from other pathogens infecting the same host. This co existence has even increased the problem of crop loss and ecnomic crisis.

In order to meet the rising demand for food in terms of both quality and quantity, plant protection in general and crop protection against plant diseases in particular are obviously important. Around 20 to 40% of worldwide agricultural production is lost as a result of direct yield losses brought on by pathogens, animals, and weeds. Crop losses brought on by pests and pathogens can be direct or indirect; they come in many forms, some of which have short-term and some long-term effects. The main goal of plant protection at that time was to shield crops from production losses brought on by both biological and non-biological factors. The issue is still difficult today as it was in the 20th century, and the limited environmental, economic, and social leeway creates further complication.

Presently it is estimated that postharvest fruit loss caused by phytopathogen fungus accounts for more than 50% of all agricultural fruit production (Zhang *et al*., 2017). Fresh fruit and vegetable post-harvest losses in India range from 4.61 to 15.9%. (Jha *et al.,* 2015). In a study conducted by Savary *et al*., (2019) they documented the yield loss due to various pathogens and pests in important food crops like wheat, rice, maize, potato and soybean worldwide. The estimated results showed that 21.5% loss in wheat crop caused mainly due to diseases like leaf rust (*Puccinia triticina*), Fusarium head blight (FHB)/scab (*Fusarium graminearum*), tritici blotch (*Septoria tritici*), stripe rust (*Puccinia striiformis* f. sp. *tritici*), spot blotch (*Cochliobolus sativus*), tan spot (*Pyrenophora tritici-repentis*) and powdery mildew (*Erysiphe graminis*).

In rice the loss was upto 30% caused by diseases like sheath blight caused by *Rhizoctonia solani*, blast caused by *Magnoporthe oryzae*, brown spot caused by *Cochliobolus miyabeanus* and bacterial blight caused by *Xanthomonas oryzae* pv.*oryzae*. Maize 22.5% yield loss was recorded which was due to diseases such as Fusarium and Gibberella stalk rots (*Fusarium* graminearum), northern leaf blight (*Exserohilum turcicum*), Fusarium and Gibberella ear rots, anthracnose stalk rot (*Colletotrichum graminicola*) and southern rust(*Puccinia sorghi*). Pathogens like *Phytopthora infestans* causing late blight, brown

rot caused by *Ralstonia solanacearum*, *Alternaria solani* which causes early blight and cyst nematode reduces 17.2% yield loss in potato. 21.4% yield loss was observed in soybean due to pathogens causing white mould (*Sclerotinia sclerotiorum*), soybean rust (*Phakopsora pachyrhizi*), Cercospora leaf blight (*Cercospora kikuchii*), brown spot (*Septoria glycines*) and charcoal rot (*Macrophomina phaseolina*)

Fungal Diseases and their Management

Few species (less than 10% of all recognised fungi) of the known fungal species are stringent saprophytes, indicating they cannot colonise plants. An even smaller portion of these plant immigrants are phytopathogenic fungi. Phytopathogenic fungi, however, not only consistently and substantially decrease crop yield annually, but they are the primary cause of disastrous agricultural plant epidemics among phytopathogens. Due to these issues of practical importance, scientists, plant breeders, and farmers all combat phytopathogenic fungus (Gonzalez-Fernandez *et al*., 2010).

1. Cultural methods for the control of plant fungal diseases:

i) Crop rotation

The growing of economic plants in repeating succession and in a specific order on the same piece of land is known as crop rotation, as opposed to a one-crop system, which typically lacks a clear strategy. Crop rotation involving maize, soybean, and wheat has reduced *F. graminearum* infection in soybean (Marburger *et al.,* 2015).

ii) Irrigation

The cultivator has a variety of options for water management, including timing, frequency, volume and manner of irrigation, to lower both foliar and soil-borne disease. Numerous times, knowing the needs for aeration and moisture in each biotic element contributing to the disease, enables the selection of a compromise watering schedule, which agricultural production that is reasonable or ideal yet nonetheless less conducive to the spread of disease. Consequently, the ideal irrigation schedule for a crop in a soil that is not infected may be different from that in a pathogen-infested soil. Gummy stem blight (*Didymella bryoniae*) and downy mildew (*Pseudoperonospora cubensis*) are some of the devastating diseases of watermelon which could be possible influenced by changing the irrigation schedule (dos Santos *et al.,* 2013)

iii) Soil solarisation

The soil is heated by sun energy in this preplanting cultural practise for soil deinfestation. The soil is heated by covering it with clear plastic sheeting at the right times of year, which also controls numerous pathogens and weeds. The dominant species of *Fusarium*

such as *F. solani, F. oxysporum, F. pseudograminearum, F. moniliforme* and *F. sambucinum has shown to have reduced population density under this method (*Saremi *et al.,* 2011*).*

2. Chemicals for the control of plant fungal diseases:

Chemicals are a crucial component of successful integrated pest management (IPM) programmes for controlling plant diseases. In the middle of the 1800s, lime sulphur and Bordeaux combination were introduced, and in the first half of the 1900s, fungicides with various sites of action and protective and contact characteristics against a number of target sites in fungal metabolism played a significant role. In the 1960s, fungicides that block a particular target site were developed. Numerous specialised fungicides were widely used up until recently due to their wide application windows, systemic activity, and protective and curative characteristics (Morton and Staub 2008).

Based on the mode of action commercial fungicides has been classified into various groups such as Sterol biosynthesis inhibitors (SBIs), quinone outside inhibitors (QoI), Dithiocarbamates, Benzimidazoles and thiophanates, Succinate dehydrogenase inhibitors (SDHIs), Chloronitriles, Phenylamides, Carboxylic acid amides (CAAs), Anilinopyrimidines etc. (Hirooka and Ishii 2013). Chemical fungicides are mostly used in commercial agriculture to protect crop plants from fungal infections by obliterating and suppressing their cells and spores. Their low cost and ease of usage, however, lead to their overuse or repeated applications (Youssef *et al*., 2019). This improper or excessive use of fungicides has had a negative impact on the environment, human and animal health, and beneficial biological systems. Furthermore, it is getting harder and harder to cure plant fungal diseases due to the advent of resistant strains of fungus phytopathogens.

3. Biological control of plant fungal diseases:

Biological control of plant diseases is the concealment of populaces of plant pathogens by living organisms (Heimpel and Mills 2017). The term "biological control" refers to the intentional use of biotic organisms that have been introduced into an area or that already exist there, such as disease-resistant host plants, to reduce the activity and population of one or more plant diseases (Pal and Gardener, 2006). Because microbial biopesticides are a crucial and invaluable component of integrated pest management (IPM), there is a growing interest in selecting them as the basis for the development of biological control agents (BCAs) (Matyjaszczyk 2015). Although microorganisms (bacteria, fungi, and viruses) are currently financially available to manage plant diseases and pests (Montesinos and Bonaterra 2017), the effectiveness of the biological products may change between preliminary results or deteriorate in field conditions (Sundin *et al.,* 2009).

Wide array of fungi and bacterial has been used as a biocontrol agent for suppressing the fungal diseases. The effective implementation of biocontrol can be credited with an array of elements, such as the microbial inoculants' rhizosphere competence, interactions with the natural microbiota, the ability to compete for nutrients, adaptation to changes in the environment, and increased host plant defence against pathogens (Pal and Gardener 2006). In addition to being incredibly helpful for managing disease, biological control is also extremely vital in establishing an eco-friendly environment. The use of biological management is crucial for controlling plant diseases without harming the native flora and fauna. It additionally boosts soil fertility (Ghorbanpour *et al.,* 2018).

Some of the bacteria listed below has been used as a bacterial biocontrol agents which are *Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Bradyrhizobium, Frankia, Pantoea, Pseudomonas, Rhizobium, Serratia, Stenotrophomonas, Streptomyces* and *Thiobacillus* (Tariq *et al.,* 2020). Increasing the activity of defense-related enzymes like peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase (PAL), producing antagonistic compounds, and altering specific root exudates like polysaccharides and amino acids are all ways that endophytic bacteria like *Bacillus* spp. and *P. fluorescens* can induce systemic resistance in plants against pathogens (Table 1).

A vast majority of the sections of fungi are physiologically and meticulously organised to prevent the growth of other pathogenic fungi that are detrimental to the advancement and development of plants. The creation of fungal strains as biocontrol agents for plant diseases has received a lot of attention. Those from the genus *Trichoderma* have been examined the most frequently (Table 2). *Penicillium, Gliocladium, Aspergillus* and *Saccharomyces* are also used as biocontrol agent against some of the major class of fungus like oomycetes and ascomycetes (Pal and Gardener 2006). Biofumigation, use of essential oil, utilization of antagonistic micro-organisms in suppressive soil and botanical fungicides are some of the approaches of biological control.

Table 1: Beneficial bacterial strains which act as biocontrol agents against phytopathogenic fungi

S.No	Bacterial Strains	Test Plant/Disease	Target Pathogen
1	*Bacillus subtilis*	Oil seed rape	*Sclerotinia sclerotiorum*
2	*Bacillus thuringiensis*	*Brassica campestris* L.	*Sclerotinia sclerotiorum*
3	*Pseudomonas fluorescens*	Apple/Mucor rot	*Mucor piriformis*
4	*Rhizobium japonicum*	Soybean/Root rot	*Fusarium solani; Macrophomina phaseolina*

5	*Bacillus licheniformis*	Pepper	*Phytophthora capsici*

Source: Tariq *et al.,* 2020

Table 2: Beneficial fungal strains which act as biocontrol agents against phytopathogenic fungi

S.No	Fungal Strains	Test Plant/Disease	Target Pathogen
1	*Penicillium oxalicum*	Tomato/wilt	*Fusarium oxysporum* f. sp. *lycopersici*
2	*Trichoderma atroviride*	Beans	*Botrytis cinerea*
3	*Trichoderma* spp.	Tobacco/root rot	*Rhizoctonia solani*
4	*Trichoderma asperellum*	Onion	*Sclerotium cepivorum*
5	*Trichoderma harzianum*	Rice/brown spot	*Bipolaris oryzae*

Source: Tariq *et al.,* 2020

4. Agriculture nanotechnology for the control of fungal diseases:

The biological effects of already-in-use antimicrobial agents are being rediscovered through nanotechnology research by manipulating their size to alter their effect. Pathogenic bacterial, fungal, and viral organisms were controlled using a variety of inorganic and organic antimicrobial nanoparticles (Mohamed *et al.*, 2021). Products made with nanomaterials, such as nano-fertilizers or nano-pesticides, have been used into agricultural practises recently. Nanoparticles, particularly metallic ones, have recently been created using biological resources like microbes, plant extracts, marine organisms, and microfluids. The most stable, cost-effective, and environmentally friendly nanoparticles are those produced using "green synthesis" employing primary and secondary metabolites of plant extracts (Baky and Amara 2021).

Silver nanoparticles, zinc oxide nanoparticles, gold nanoparticles and copper nanoparticles are some examples of nanoparticles that represent green nanotechnology application in fungal management along with their targeted pathogens. Krishnaraj *et al.,* (2012) reported the antifungal activity of *Acalypha indica* leaf extract for rapid synthesis of silver nanoparticles at a concentration of 15 mg against a number of phytopathogenic fungi, including *Rhizoctonia solani, Sclerotinia sclerotiorum, Alternaria alternata, Botrytis cinerea, Macrophomina phaseolina* and *Curvularia lunata*. The potential of these nanoparticles to suppress *Fusarium graminearum* by limiting its mycelial growth and mycotoxin production was proven by Lakshmeesha *et al.,* (2019), who reported biofabrication of ZnO NPs utilising buds extract of *Syzygium aromaticum* flowers. Jayaseelan *et al.,* (2013) in their study stated the role

of gold nanoparticles synthesized by the seeds of *Abelmoschus esculentus* aqueous extract which had fungicidal effects on important phytopathogens *Aspergillus niger, Aspergillus flavus* and *Puccinia graminis* var. *tritci. Citrus medica* was used in the green synthesis of copper nanoparticles by Shende *et al*., (2015), who also demonstrated the inhibitory effects of these nanoparticles on *Fusarium oxysporum, Fusarium culmorum* and *Fusarium graminearum.*

The use of ultraviolet light for the eradication of plant fungal infections is among other management strategies that were considered to be of future relevance. AMF's (arbuscular mycorrhizal fungi) impact on plant defence mechanisms against fungi, Mycoviruses to limit the virulence of phytopathogenic fungi, homoeopathy and tea derived from herbal flowers for controlling phytopathogenic fungi, increasing plant resistance to fungal illnesses, and fungal cell deactivation and evacuation employing ghost techniques are some other methods (Baky and Amara 2021).

Bacterial Diseases and their Management

1. Cultural methods

i. Crop rotation

Crop rotation is a cultural method of disease management which is based on growing a series of crops in the successive seasons in order to minimise the host range of the pathogens and starve them to death. The brown rot of potato caused by *Ralstonia solanacearum* was found to be controlled by crop rotation with cabbage or lablab or cereals like wheat (Mwaniki, 2017).

ii. Selection of resistant varieties

The resistant varieties which can withstand the pathogen attack can be selected to avoid the infection caused by the pathogens. Till now many resistant varieties were developed and were released in India. Some of the varieties resistant to bacterial blight of cotton caused by *Xanthomonas campestris* pv. *malvacearum* are MCU 10, L 389, L 604, LAHH 4, Arogya (Waghmare, 2016) and the variety IR24 is resistant to bacterial blight of rice cause by *Xanthomonas oryzae* pv. *oryzae* (Zhang, 2022). The varieties reported to be resistant against *Pseudomonas rubrilineans* causing red stripe of sugarcane were Co cultivars 6805, 7537, 7202 (Singh, 2018a).

iii. Inoculum free seeding or planting material

The first and foremost step in disease management is to select the disease free seeds or planting material. The tundu disease of wheat caused by the combination of *Clavibacter tritici* and *Anguina tritici* produces galls which can be easily mixed with the seeds and have to be removed before sowing the seeds to ensure that the disease

free seeds were sown. Likewise, the black rot of crucifers caused by *Xanthomonas campestris* pv. *campestris* is a seed borne disease which can be controlled by sowing the seeds after seed health testing in laboratories (Gitaitis & Walcott, 2007).

iv. Crop sanitation

Crop sanitation is an important practice for disease management where the suspected diseased plant parts are removed continuously during the cropping season. Rouging of the infected plant parts and the weed hosts come under the crop sanitation. For e.g. In the case of citrus canker and the citrus greening diseases which is caused by the bacteria, *Xanthomonas axonopodis* pv. *citri* (Singh, 2018b) and *Candidatus liberobacter asiaticum* (Gottwald, 2007) respectively, the infected plants or the plant parts should be removed immediately from the field to control the spreading of the disease.

v. Amendment of organic manures to the soil

One of the most cost effective methods of disease management is amending soil with the organic manures like green manure crops, farmyard manure, mustard manure, soybean manure, swine manure, fish manure etc. These type of manures produce toxic compounds which have the ability to kill the pathogens (Lazarovits *et al*., 2001). Apart from the disease management, these manures can provide nutrition to the plants and also it can alter the physical and chemical characteristics of the soil (Akanmu *et al*., 2021). For e.g., the potato plants when treated with mustard manure showed reduced disease incidence of common scab disease caused by *Streptomyces scabies* (Gharate *et al*., 2016).

vi. Adjustment in the date of sowing

The date of sowing of the crops can be adjusted to avoid the critical stage of infection and the favourable environmental conditions so that the plant escapes disease. In case of cotton, it was observed that yield loss occurs during late planting and yield is increased during early planting (Afzal *et al*., 2020). And so, the early planted cotton escapes from the bacterial blight disease caused by *Xanthomonas axonopodis* pv. *malvacearum* (Singh, 2018b).

vii. Management of acidity / alkalinity of the soil

Many pathogens can grow in the type of soils in which their host plants grow. But some pathogens are sensitive to the pH and cannot grow when the pH of the soil is manipulated. For e.g., the potato scab pathogen, *Streptomyces scabies* is sensitive when the pH is lowered to 5.2 (Hamedo & Makhlouf, 2013) and so application of gypsum or sulphur to lower the pH below 5.2 effectively reduces the severity of the disease (Goswami & Mishra, 2022).

viii. Management of host nutrition

The nutrients supplied to the plants also plays a major role in plant resistance or susceptibility. High nitrogen sometimes induce the vegetative growth of the plants and ir favours the disease development. The bacterial blight and bacterial streak diseases of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Chao *et al*., 2015) and *X. oryzae* pv. *oryzicola* (Singh, 2018b) and bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* infects the plants which were provided with high levels of nitrogen (Hoffland *et al*., 2000). The disease, black rot of crucifers caused by *X. campestris* pv. *campestris* can be reduced by applying boron alone or with nitrogen (Singh, 2018b).

2. Mechanical methods - Sieving of galls and water floating / sedimentation methods

The seed galls produced in the ear cockle / tundu disease of wheat can be easily mixed with the seeds and act as inoculum for the next season. It should be removed before sowing and the methods used to separate galls from seeds are sieving of seeds, using brine solution or water floatation methods. In water floatation method, the seeds are soaked for 10 – 15 minutes in water and the cockles which are light weighted will float on the top and can be removed. Another method is the seeds are soaked in 20 – 30 % brine/ common salt solution and the cockles are floated on the top which can be removed (Navik & Varshney, 2017).

3. Physical methods

i. Hot water treatment

Hot water treatment is usually followed to eradicate the seed borne inoculum and thereby controlling seed borne pathogens before sowing. The bacterial blight pathogen *X. oryzae* pv. *oryzae* can be controlled by hot water treatment at 52 - 55^{0}C for 20 – 30 minutes (Saha *et al*., 2015). *X. campestris* pv. *campestris* causes black rot of cabbage can be controlled by hot water treatment at 50^{0}C for 20 – 30 minutes. Ratoon stunting disease of sugarcane caused by *Clavibacter xyli* ssp. *xyli* can be controlled by treating the setts in hot water at 50^{0}C for 3 hours (Chaube and Pundhir, 2005).

ii. Hot air treatment

This method is also used to reduce the seed borne inoculum and seed injury is less in this method compared to hot water treatment. The hot air treatment of sugarcane stalks at 54^{0}C for 8 hours is followed to control ratoon stunting disease (Chaube and Pundhir, 2005).

iii. Aerated steam therapy

Aerated steam therapy is mainly followed to control the seed borne pathogens and

it is safer than hot water treatment (Kiran *et al*., 2021). This method is followed to control the sugarcane ratoon stunting disease and sugarcane grassy shoot disease if the stalks are treated at 50^0C for 1 hour (Parameswari & Viswanathan, 2021). This method is also followed for the control of citrus greening disease (Chaube and Pundhir, 2005).

4. Biological methods

i. Application of biocontrol agents

Biological control using biocontrol agents is another effective method of disease management in which the beneficial organisms are used to suppress the harmful pathogens in the soil, seed surface, foliar parts etc. The mechanism of biocontrol agents are antibiosis, competition for nutrients, hyperparasitism and induction of systemic resistance of the plants. For e.g., seed treatment with *Pseudomonas fluorescens* P32 strain was found to reduce the incidence of *Ralstonia solanacearum* in tomato (Zhou *et al*., 2005). Seed treatment with *P. fluorescens* and *P. putida* also suppressed the bacterium *Xanthomonas axonopodis* pv. *malvacearum* causing bacterial blight of cotton in seedling stage. Bacteriophages can also be used as biocontrol agents. For e.g., bacteriophages belonging to *Caulimovirales* was found to act against the black leg pathogen *Erwinia carotovora* ssp. *carotovorum* (Singh, 2018a).

ii. Application of plant extracts

Plant extracts and essential oils can be successfully exploited in disease management as it contains anti-fungal, anti-bacterial compounds and also it is free from residues and are eco-friendly. These plant extracts also serves as a nutrient source to the plants when degraded. It was found that 15% *Datura alba* plant extract suppressed *X. axonopodis* pv. *malvacearum* which causes bacterial blight of cotton (Javed *et al*., 2013). The crude extract of *Sapium baccatum* was found to be very active against bacterial wilt of tomato caused by *Ralstonia solanacearum* (Vu *et al*., 2017).

5. Chemical methods

Antibiotics can be a good method to control bacterial pathogens and also a reliable one. Some of the antibiotics are streptomycin, tetracycline, agrimycin, oxymycin, terramycin etc. Streptomycin is a most common bacterial antibiotic used to control many plant pathogens like bacterial blight of rice and cotton. Seed treatment using agrimycin (0.01%) was effective against *X. campestris* pv. *campestris* which cause black rot of crucifers (Singh, 2018a). Seed treatment of potato tubers with thaibendazole 1% was found to control soft rot of potato caused by *E. caratovora* var. *atroseptica* (Chaube and Pundhir, 2005).

6. Biotechnological approaches

Recently biotechnological methods of disease management are gaining importance as it provides complete control of the pathogen on which the method is targeted. Biotechnological methods include marker assisted selection, gene pyramiding, genome editing and other transgenic methods. A notable variety pusa basmathi was backcrossed by marker assisted breeding to incorporate bacterial blight resistance genes Xa13 and Xa21. The resistant varieties PB1121 and PB6 developed by this method were reported to be completely resistant to bacterial blight of rice (Ellur *et al.*, 2016).

Viral Diseases and their Management

1. Cultural methods

i. Crop rotation

Crop rotation can be followed for viral disease management. Cucumber green mottle mosaic virus (CGMMV) infecting watermelon is a soil borne virus and when rotation between watermelon and rice was followed, a drastic reduction in the disease was observed in fields (Park *et al.*, 2010).

ii. Selection of resistant varieties

The blackgram variety VBN6 is resistant to *Blackgram yellow mosaic virus* can be successfully exploited against the virus under field conditions (Kamesh krishnamoorthy *et al.*, 2021). The variety Parbhani kranti was developed especially for resistance against yellow vein mosaic of bhendi was reported to be highly resistant to the disease (Islam, 2017).

iii. Inoculum free seeding or planting material

This method is followed to ensure that the seeds were free from viruses. This is done by grow out tests (GOT). The grow out testing can be followed for *Potato virus Y*, *Bean common mosaic virus*, *Pea seed borne mosaic virus*, *Mild mosaic viruses of potato*, *Radish yellow edge virus*, etc.

iv. Crop sanitation

Most of the viruses are vector transmitted and crop sanitation and rouging should be compulsorily followed to prevent the spread of infection. *Potato virus X*, *Potato virus S* and *Potato spindle tuber viroid* are the most contagious ones and rouging should be followed to stop the disease from spreading (Chaube and Pundhir, 2005).

v. Plant spacing

Plant spacing also plays a major role in the transmission of viruses by vectors. Sometimes the crops should be planted densely or sparsely by altering the seed rate

to reduce severity of the diseases. Sparse planting favours the diseases, tomato leaf curl transmitted by *Bemesia tabaci*, cucumber mosaic transmitted by *Aphis gossypii* and groundnut rosette transmitted by *A. craccivora* and hence dense planting is followed to reduce the disease incidence (Singh, 2018b).

vi. Management of host nutrition

Excessive application of nitrogen can help plants to produce dense canopy which attracts the insects which transmits the viruses. Hence, recommended amount of nitrogen should be used to limit the insect transmission.

vii. Intercropping

Intercropping of host crops with other non host crops can reduce the incidence of viruses. It was found that the blackgram intercropped with sorghum had shown lesser yellow mosaic incidence compared to the disease incidence in blackgram alone (Swathi *et al.*, 2019).

viii. Border crops

Border crops are grown for the purpose of protecting the main crop from insect vectors. This method restricts the movement of insect vectors into the main crop. Border cropping of sorghum with blackgram as main crop controls yellow mosaic incidence of blackgram (Swathi *et al.*, 2019).

2. Physical methods

i. Hot water treatment

Hot water treatment can be followed to control diseases like *Sugarcane mosaic virus*, *Sugarcane steak mosaic virus* (SCSMV) etc. HWT recommended for the control of sugarcane mosaic virus was 52^{0}C for 30 minutes (Islam, 2017) and for SCSMV was 53^{0}C for 10 minutes (Damayanti & Putra, 2010). HWT for sugarcane grassy shoot disease was recommended as 50^{0}C for 1 hour (Tiwari and Rao, 2023). The seed borne virus, tomato mosaic can be controlled by HWT at 50^{0}C for 25 minutes (Singh, 2018a).

ii. Hot air treatment

Hot air treatment was given by Sinclair in 1832 for the control of seed borne smut disease of oats and barley. Later, it was found that hot air treatment of sugarcane stalks at 54^{0}C for 8 hours controls the grassy shoot disease of sugarcane (Singh and Pandey, 2012).

iii. Aerated steam therapy

The seed borne viral diseases like sugarcane grassy shoot disease can be controlled by

this method. Aerated steam therapy of single/two/three budded setts of sugarcane at 50^{0}C for 1 hour was found to be effective against sugarcane grassy shoot disease (Parameswari and Viswanathan, 2021).

iv. Moist hot air treatment

This technique is mainly followed to control sugarcane grassy shoot disease. The setts are first subjected to hot air at 54^{0}C for 8 hours, then to aerated steam at 50^{0}C for 1 hour, and finally to moist hot air at 54^{0}C for 2 hours (Singh and Pandey, 2012).

v. Use of sticky traps

Direct vector control is accomplished by using sticky traps. This sticky traps is coloured so that they attract the insect vectors towards them and they stick to the traps and cannot move away. Thus transmission of viruses can be controlled by using sticky traps. The sticky traps may be yellow or blue in colour. The former is used for all sucking pests (aphids, whiteflies etc.) and the latter is especially used for attracting thrips. This is a common method for preventing the potato virus and CMV in peppers transmitted by aphids (Chandi, 2021).

3. Biological methods

Anti-viral extracts of many plants like coconut, sorghum, bougainvillea, *Prosopis julifera*, *Cyanadon dactylon* etc. are known to control viral diseases. The whole plant extract (WPE) of *Haplophyllum tuberculatum*, a medicinal plant was reported to have antiviral properties and was effective against *Tobacco mosaic virus* (Abdelkhalek *et al*., 2020). The root extracts of *Mirabilis jalaba* at 6% were found to be active against *Peanut bud necrosis virus* (Sangeetha, 2020). Azadirachtin, an active compound present in neem has an ability to repel insects and it is used to control *Barley yellow dwarf virus* transmitted by aphids (Chandi, 2021).

The biocontrol agents like *Bacillus* sp. does not have direct effect on the viral pathogens but they have the property of enhancing the induced systemic resistance (ISR) in the plants. The biocontrol agent, *B. subtilis* subsp. *subtilis* when applied to *Arabidopsis thaliana* plants, induces resistance in the plants against *Cucumber mosaic virus* (Elsharkawy *et al*., 2022). The foliar application antagonistic *B. subtilis* strain HA1 was reported to have a positive effect on controlling *Tobacco mosaic virus* infection in tomato (El-Gendi *et al*., 2022).

4. Chemical methods

There is no viricide identified so far to kill the viruses. But, the vectors which transmit them can be controlled by chemical methods. The long lasting insecticide treated nets (LLITNs) were used to control the aphid (*Aphis gossypii*, *Myzus persicae*)

population and it controlled *Cucumber aphid-borne yellow virus* (Dader *et al*., 2015). The insecticide, imidachloprid can be used to control the major hemipteran sucking pests like aphids, thrips, whiteflies, leafhoppers and planthoppers transmitting many economically important diseases like *Tomato spotted wilt virus*, *Peanut bud necrosis virus*, *Potato leaf roll virus*, *Barley yellow dwarf virus* etc. (Castle *et al*., 2009).

5. Biotechnological approaches

The biotechnological approaches for viral disease management includes transgenic approaches - pathogen derived resistance like coat protein mediated resistance, movement protein mediated resistance, satellite mediated resistance, replicase mediated resistance etc., non pathogen derived resistance like resistance genes, ribosomal inactivating proteins, antiviral plantibodies etc. and tissue culture methods for virus free seedlings. The transgenic papaya varieties Sunup and Rainbow were developed in 1995 to be resistant against the devastating Papaya ringspot virus using coat protein mediated resistance and it was successfully exploited in Hawaii islands (Ferreira *et al*., 2002). The cassava mosaic virus free plantings can be obtained by meristem tissue culture which was effective in controlling the disease (Deepthi and Makeshkumar, 2016).

6. Advanced methods – Nanophytovirology

Recently nanotechnology has been playing a major role in all the branches of agriculture and all other research sectors. The dsRNA technology was developed and is an effective method for viral disease management but the problem is that dsRNA is unstable and it has to be coated with nanoparticles to have successful control. The dsRNA loaded onto hydroxide nanoparticles to form dsRNA bioclay and the bioclay when applied exogenously to *Nicotiana benthamiana*, a potential control against *Bean common mosaic virus* was achieved (Worrall *et al*., 2019). It was also reported that application of nanoparticles have controlling effects on viral diseases and the mechanism of action is not clearly known. The application of silver nanoparticles was found to control *Bean yellow mosaic virus* because of the ability to attach to the glycoprotein of the viruses (Singh *et al*., 2022).

Conclusion

The development of various management strategies is crucial for combating plant diseases brought on by plant pathogens. Agronanotechnology has taken centre stage since this decade, combined with the application of biological control agents. Integrating disease resistant measures and early diagnosis can help in minimising yield loss with the development of diverse scientific procedures, which on the other hand would open the road to feed the growing global population.

References

Abdelkhalek, A., Salem, M. Z., Hafez, E., Behiry, S. I., & Qari, S. H. (2020). The phytochemical, antifungal, and first report of the antiviral properties of Egyptian *Haplophyllum tuberculatum* extract. *Biology*, 9(9), 248.

Afzal, M. N., Tariq, M., Ahmed, M., Abbas, G., & Mehmood, Z. (2020). Managing planting time for cotton production. *Cotton Production and Uses: Agronomy, Crop Protection, and Postharvest Technologies*, 31-44.

Akanmu, A. O., Babalola, O. O., Venturi, V., Ayilara, M. S., Adeleke, B. S., Amoo, A. E., ... & Glick, B. R. (2021). Plant disease management: leveraging on the plant-microbe-soil interface in the biorational use of organic amendments. *Frontiers in Plant Science*, *12*, 700507.

Castle, S., Palumbo, J., & Prabhaker, N. (2009). Newer insecticides for plant virus disease management. *Virus Research*, 141(2), 131-139.

Chandi, R. S. (2021). Integrated management of insect vectors of plant pathogens. *Agricultural Reviews*, 42(1), 87-92.

Chao, Y. U., CHEN, H. M., Fang, T. I. A. N., Bi, Y. M., Steven, R. J., Jan, L. E., & HE, C. Y. (2015). Identification of differentially-expressed genes of rice in overlapping responses to bacterial infection by *Xanthomonas oryzae* pv. *oryzae* and nitrogen deficiency. *Journal of Integrative Agriculture*, 14(5), 888-899.

Chaube, H. S., & Pundhir, V. S. (2005). *Crop diseases and their management*. PHI Learning Pvt. Ltd..

Dáder, B., Legarrea, S., Moreno, A., Plaza, M., Carmo-Sousa, M., Amor, F., ... & Fereres, A. (2015). Control of insect vectors and plant viruses in protected crops by novel pyrethroid-treated nets. *Pest Management Science*, 71(10), 1397-1406.

Damayanti, T. A., & Putra, L. K. (2010). Hot water treatment of cutting-cane infected with Sugarcane streak mosaic virus (SCSMV). *Journal of ISSAAS (International Society for Southeast Asian Agricultural Sciences)*, 16(2), 17-25.

Deepthi, D. C., & Makeshkumar, T. (2016). Elimination of cassava mosaic disease through meristem culture and field evaluation for yield loss assessment in cassava genotypes. *Journal of Root Crops*, 42(1), 45-52.

dos Santos, G. R., Leão, E. U., Gonçalves, C. G., & Cardon, C. H. (2013). Potassium fertilizer and irrigation management in the progress of fungal diseases and yield of watermelon. *Horticultura Brasileira*, *31*, 36-44.

El-Baky, N. A., & Amara, A. A. A. F. (2021). Recent approaches towards control of fungal diseases in plants: An updated review. *Journal of Fungi*, *7*(11), 900.

El-Gendi, H., Al-Askar, A. A., Király, L., Samy, M. A., Moawad, H., & Abdelkhalek, A. (2022). Foliar Applications of Bacillus subtilis HA1 Culture Filtrate Enhance Tomato Growth and Induce Systemic Resistance against Tobacco mosaic virus Infection. *Horticulturae*, 8(4), 301.

Ellur, R. K., Khanna, A., Yadav, A., Pathania, S., Rajashekara, H., Singh, V. K., ... & Singh, A. K. (2016). Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. *Plant Science*, 242, 330-341.

Elsharkawy, M. M., Elsawy, M. M., & Ismail, I. A. (2022). Mechanism of resistance to Cucumber mosaic virus elicited by inoculation with Bacillus subtilis subsp. subtilis. *Pest Management Science*, 78(1), 86-94.

Ferreira, S. A., Pitz, K. Y., Manshardt, R. I. C. H. A. R. D., Zee, F., Fitch, M., & Gonsalves, D. E. N. N. I. S. (2002). Virus coat protein transgenic papaya provides practical control of papaya ringspot virus in Hawaii. *Plant Disease*, 86(2), 101-105.

Gharate, R. A. M. A. K. A. N. T., Singh, N. A. R. E. N. D. R. A., & Chaudhari, S. M. (2016). Management of common scab (*Streptomyces scabies*) of potato through eco-friendly approach. *Indian Phytopathol*, 69(3), 266.

Ghorbanpour, M., Omidvari, M., Abbaszadeh-Dahaji, P., Omidvar, R., & Kariman, K. (2018). Mechanisms underlying the protective effects of beneficial fungi against plant diseases. *Biological Control*, *117*, 147-157.

Gitaitis, R., & Walcott, R. (2007). The epidemiology and management of seedborne bacterial diseases. *The Annual Reviews of Phytopathology*, 45, 371-397.

González-Fernández, R., Prats, E., & Jorrín-Novo, J. V. (2010). Proteomics of plant pathogenic fungi. *BioMed Research International*, *2010*.

Goswami, S., & Mishra, S. COMMON SCAB OF POTATO (*STREPTOMYCES SCABIES*): A REVIEW. *Dr. Zeenat N. Kashmiri*, 105.

Gottwald, T. R. (2007). Citrus canker and citrus huanglongbing, two exotic bacterial diseases threatening the citrus industries of the Western Hemisphere. *Outlooks on Pest Management*, 18(6), 274.

Hamedo, H. A., & Makhlouf, A. H. (2013). Identification and characterization of actinomycetes for biological control of bacterial scab of *Streptomyces scabies* isolated from potato. *Journal of Biology, Agriculture and Healthcare*, 3(13), 142-153.

Heimpel, G. E., & Mills, N. J. (2017). *Biological control*. Cambridge University Press.

Hirooka, T., & Ishii, H. (2013). Chemical control of plant diseases. *Journal of General Plant Pathology*, *79*, 390-401.

Hoffland, E., Jeger, M. J., & van Beusichem, M. L. (2000). Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. *Plant and Soil*, 218, 239-247.

Islam, W. (2017). Management of plant virus diseases; farmer's knowledge and our suggestions. *Hosts and Viruses*, 4(2), 28.

Javed, M. T., Khan, M. A., Ehetisham-ul-Haq, M., & Atiq, M. (2013). Biological management of bacterial blight of cotton caused by *Xanthomonas campestris* pv. *malvacearum* through plant extracts and homeopathic products. *Res J Plant Dis Pathol*, 1, 1-10.

Jayaseelan, C., Ramkumar, R., Rahuman, A. A., & Perumal, P. (2013). Green synthesis of gold nanoparticles using seed aqueous extract of *Abelmoschus esculentus* and its antifungal activity. *Industrial Crops and Products*, *45*, 423-429.

Jha, S. N., Vishwakarma, R. K., Ahmad, T., Rai, A., & Dixit, A. K. (2015). Report on assessment of quantitative harvest and post-harvest losses of major crops and commodities in India. *All India Coordinated Research Project on Post-Harvest Technology, ICAR-CIPHET*, *130*.

Kamesh Krishnamoorthy, K., Malathi, V. G., Renukadevi, P., Mohan Kumar, S., Raveendran, M., Manivannan, N., ... & Karthikeyan, G. (2021). Population structure of whitefly (*Bemisia tabaci*) and the link between vector dynamics and seasonal incidence of yellow mosaic disease in blackgram (*Vigna mungo*). *Entomologia Experimentalis et Applicata*, 169(4), 403-412.

Kiran, R., Akhtar, J., & Kumar, P. (2021). Thermotherapy: a non-chemical option for managing seed-borne bacterial diseases. *Agri-India TODAY*, 1(3), 1-5.

Krishnaraj, C., Jagan, E. G., Ramachandran, R., Abirami, S. M., Mohan, N., & Kalaichelvan, P. T. (2012). Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism. *Process biochemistry*, *47*(4), 651-658.

Kumar, S., Thakur, M., & Rani, A. (2014). *Trichoderma:* Mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. *African journal of agricultural research*, *9*(53), 3838-3852

Lakshmeesha, T. R., Kalagatur, N. K., Mudili, V., Mohan, C. D., Rangappa, S., Prasad, B. D., & Niranjana, S. R. (2019). Biofabrication of zinc oxide nanoparticles with *Syzygium aromaticum* flower buds extract and finding its novel application in controlling the growth and mycotoxins of *Fusarium graminearum*. *Frontiers in microbiology*, *10*, 1244.

Lazarovits, G., Tenuta, M., & Conn, K. L. (2001). Organic amendments as a disease control strategy for soilborne diseases of high-value agricultural crops. *Australasian Plant Pathology*, *30*, 111-117.

Marburger, D. A., Venkateshwaran, M., Conley, S. P., Esker, P. D., Lauer, J. G., & Ané, J. M. (2015). Crop rotation and management effect on Fusarium spp. populations. *Crop Science*, *55*(1), 365-376.

Matyjaszczyk, E. (2015). Products containing microorganisms as a tool in integrated pest management and the rules of their market placement in the European Union. *Pest management science*, *71*(9), 1201-1206.

Mohamed, A. A., Elshafie, H. S., Sadeek, S. A., & Camele, I. (2021). Biochemical characterization, phytotoxic effect and antimicrobial activity against some phytopathogens of new Gemifloxacin schiff base metal complexes. *Chemistry & Biodiversity*, *18*(9), e2100365.

Montesinos, E., & Bonaterra, A. (2017). Pesticides, microbial In Reference module in life sciences.

Morton, V., & Staub, T. (2008). A short history of fungicides. *APSnet Features*, *308*, 1-12.

Mwaniki, P. K. (2017). Impact of crop rotation sequences on potato in fields inoculated with bacterial wilt caused by *Ralstonia solanacearum*, 12(14), 1226-1235.

Navik, O. S., & Varshney, R. (2017). Field pests of wheat and their management. *Wheat a Premier Food CroP*, 322.

Pal, K. K., & Gardener, B. M. (2006). Biological control of plant pathogens.

Parameswari B, K. N., & Viswanathan, R. (2021). Sugarcane: Ratoon stunting and Grassy shoot. Today & Tomorrow's Printers and Publishers, New Delhi, India.

Park, J. W., Jang, T. H., Song, S. H., Choi, H. S., & Ko, S. J. (2010). Studies on the soil transmission of CGMMV and its control with crop rotation. *The Korean Journal of Pesticide Science*, 14(4), 473-477.

Ristaino, J. B., Anderson, P. K., Bebber, D. P., Brauman, K. A., Cunniffe, N. J., Fedoroff, N. V., & Wei, Q. (2021). The persistent threat of emerging plant disease pandemics to global food security. *Proceedings of the National Academy of Sciences*, *118*(23), e2022239118.

Saha, S., Garg, R., Biswas, A., & Rai, A. B. (2015). Bacterial diseases of rice: an overview. *J Pure Appl Microbiol,* 9(1), 725-736.

Sangeetha, B., Krishnamoorthy, A. S., Renukadevi, P., D Malathi, V. G., & Sharmila,

A. D. J. S. (2020). Antiviral potential of *Mirabilis jalapa* root extracts against groundnut bud necrosis virus. *Journal of Entomology and Zoology Studies*, 8(1), 955-961.

Saremi, H., Okhovvat, S. M., & Ashrafi, S. J. (2011). Fusarium diseases as the main soil borne fungal pathogen on plants and their control management with soil solarization in Iran. *African Journal of Biotechnology*, *10*(80), 18391-18398.

Savary, S., Ficke, A., Aubertot, J. N., & Hollier, C. (2012). Crop losses due to diseases and their implications for global food production losses and food security. *Food security*, *4*(4), 519-537.

Shende, S., Ingle, A. P., Gade, A., & Rai, M. (2015). Green synthesis of copper nanoparticles by Citrus medica Linn.(Idilimbu) juice and its antimicrobial activity. *World Journal of Microbiology and Biotechnology*, *31*, 865-873.

Singh, R. S. (2018a). *Plant diseases*. Oxford and IBH Publishing.

Singh, R. S. (2018b). *Introduction to principles of plant pathology*. Scientific International (Pvt.) Ltd.

Singh, R., Kuddus, M., Singh, P. K., & Choden, D. (2022). Nanotechnology for Nanophytopathogens: From Detection to the Management of Plant Viruses. *BioMed Research International*, 2022.

Singh, V. K., & Pandey, P. (2012). Physical methods in management of plant diseases. *Plant Dis*, 10(1).

Sundin, G. W., Werner, N. A., Yoder, K. S., & Aldwinckle, H. S. (2009). Field evaluation of biological control of fire blight in the eastern United States. *Plant Disease*, *93*(4), 386-394.

Swathi, P., Basu, B. J., Rao, N. S., & SaidaNaik, V. (2019). Management of yellow mosaic virus in blackgram through agronomic practices. *Journal of Entomology and Zoology Studies*, 7(3), 1570-1573.

Tariq, M., Khan, A., Asif, M., Khan, F., Ansari, T., Shariq, M., & Siddiqui, M. A. (2020). Biological control: a sustainable and practical approach for plant disease management. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *70*(6), 507-524.

Tiwari, A. K., & Rao, G. P. (2023). Production of sugarcane grassy shoot disease free setts by using hot water treatment. *Phytopathogenic Mollicutes*, 13(1), 73-74.

Vu, T. T., Kim, H., Tran, V. K., Vu, H. D., Hoang, T. X., Han, J. W., ... & Kim, J. C. (2017). Antibacterial activity of tannins isolated from *Sapium baccatum* extract and use for control of tomato bacterial wilt. *PLoS One*, 12(7), e0181499.

Waghmare, V. N. (2016). Cotton improvement in India. *Cotton technology exchange program in SAARC region*, 61-78.

Worrall, E. A., Bravo-Cazar, A., Nilon, A. T., Fletcher, S. J., Robinson, K. E., Carr, J. P., & Mitter, N. (2019). Exogenous application of RNAi-inducing double-stranded RNA inhibits aphid-mediated transmission of a plant virus. *Frontiers in plant science*, 10, 265.

Youssef, K., de Oliveira, A. G., Tischer, C. A., Hussain, I., & Roberto, S. R. (2019). Synergistic effect of a novel chitosan/silica nanocomposites-based formulation against gray mold of table grapes and its possible mode of action. *International Journal of Biological Macromolecules*, *141*, 247-258.

Zhang, J., Feng, X., Wu, Q., Yang, G., Tao, M., Yang, Y., & He, Y. (2022). Rice bacterial blight resistant cultivar selection based on visible/near-infrared spectrum and deep learning. *Plant Methods*, 18(1), 1-16.

Zhang, L., Lan, R., Zhang, B., Erdogdu, F., & Wang, S. (2021). A comprehensive review on recent developments of radio frequency treatment for pasteurizing agricultural products. *Critical Reviews in Food Science and Nutrition*, *61*(3), 380-394.

Zhou, H., Wei, H., Liu, X., Wang, Y., Zhang, L., & Tang, W. (2005). Improving biocontrol activity of Pseudomonas fluorescens through chromosomal integration of 2, 4-diacetylphloroglucinol biosynthesis genes. *Chinese Science Bulletin*, 50, 775-781.

4 Plant Disease Epidemiology

Nimmala Sree Valli[1] and Rajkumari Jyotika[1]

[1]Ph.D. Scholar, Department of Plant Pathology, Agricultural College and Research Institute, Coimbatore, Tamilnadu Agricultural University, Tamil Nadu - 641003)

Abstract

The study of diseases in populations, especially in plant populations, is known as epidemiology. It is a quantitative field with solid theoretical foundations and real-world applications. Plant disease outbreaks have had a significant influence on populations of forest trees, agricultural and horticultural crops, and other plant species, with significant socioeconomic and political impacts. Methodologies for evaluating disease progression in time and space, the derivation of critical factors defining the rate of epidemic progress, and the degree of control required for disease management are all part of an epidemiologist's toolbox. By introducing genetic variation in the host and pathogen, the role of disease vectors in transmission, and higher level interactions involved in biological regulation, the intricacy of biotic interactions controlling plant epidemics is demonstrated. It is determined how important disease is to communities of wild plant life. Some modifications to the epidemiological toolbox are suggested in light of the growing knowledge of the landscape connectivity of crop plants, natural communities, and invasive species.

Keywords: Epidemic, Epidemiology, Forecasting, Disease, Pathogen

Introduction

Epidemic is defined as the phenomenon of rapid spread of a pathogen and affects many individual within a population over a relatively large area within a relative short period of time. It is a dynamic change of plant disease in space and time. The study of a pathogen's rate of multiplication, which determines its ability to transmit

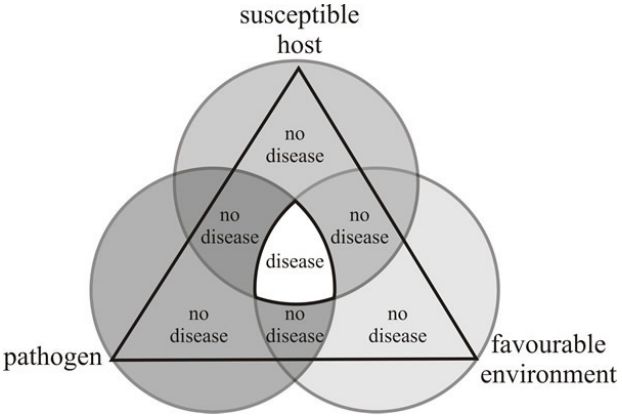
disease throughout a population of plants, is known as epidemiology or epiphytology. Epidemiology is concerned with both population genetics of the host resistance and the pathogen population's capacity for evolution to develop pathogen races that may be more resistant to pesticides or more virulent to host types. Epidemiology, which has implications in human, animal, and plant diseases, is the study of how disease spreads among populations. Epidemiology has evolved into a quantitative discipline for plant diseases with the goals of characterising, defining, and predicting epidemics, as well as intervening to reduce the effects on plant populations. Although the population level is the primary focus of epidemiology, it is frequently required to scale up to the individual plant/cellular level and down to the community/landscape level in order to recognise the system hierarchies that are present.

Table 1: Some important historical epiphytotics

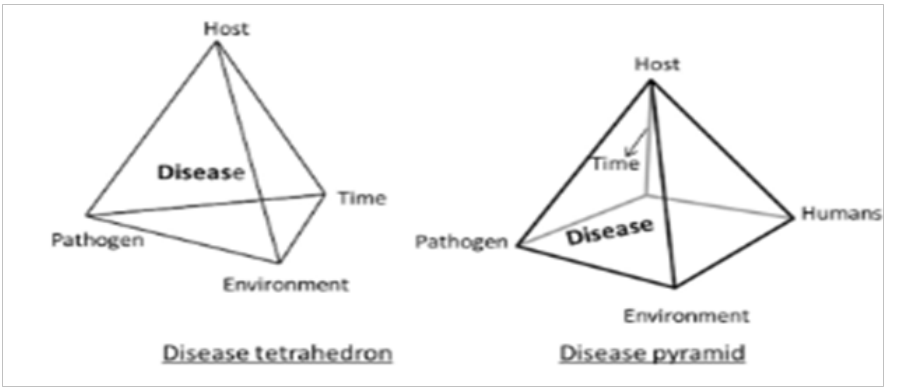
Year	Disease	Causal Organisms	Locality
1845	Late blight of potato (The Irish Famine)	*Phytophthora infestans*	Ireland
1870	Coffee Rust	*Hemileia vastatrix*	Sri Lanka
1878	Downy mildew of grapevines	*Plasmopara viticola*	France
1900	Lethal yellow of cocoa	MLO	Cuba
1904	Chestnut blight	*Endothea parasitica*	America
1916	Wheat Rust	*Puccinia tritici*	USA, Canada
1921	Banana bunchy top	MLO	Australia
1930	Sigatoka disease of Banana	*Mycosphaerella musicola*	America
1936	Red rot of Sugarcane	*Colletotrichum falcatum*	Uttar Pradesh (India)
1943	Brown spot of Rice (The Great Bengal Famine)	Drechslera oryzae	West Bengal (India)
1951	Bacterial blight of Rice	*Xanthomonas campestris* pv. *campestris*	Maharashtra (India)
1969	Southern corn blight	*Helminthosporium maydis* race T	USA
1984	Apple Scab	*Venturia inaequalis*	Jammu & Kashmir (India)
1999	Rice tungro disease	*Rice tungro bacilliform* & *Spherical virus*	Punjab (India)

The elements of an epidemics

Plant disease epidemics develop as an outcome of interaction between susceptible host plant, a virulent pathogen and favourable environmental conditions. When favourable interaction occur between these three components, it is defined as **disease triangle**. In disease triangle, host factors includes its level of susceptibility or genetic resistance of host, degree of genetic uniformity of host, age of host plant and type of crop; pathogen factors include aggressiveness, amount of inoculum, level of virulence, type of reproduction and mode of spread of pathogens and environmental factors includes optimum moistures, temperature, soil pH, light, oxygen and carbon-dioxide concentration for disease development.



The interaction of these three components in disease development is affect by fourth and fifth components: - time and humans. The effect of time on disease development include the duration and frequency of favourable rain and temperature, time of the year (i.e., the climatic conditions and stage of growth when host and pathogen may co-exist), duration of disease infection cycle and time of appearance of vectors. Human activities also interfere with epidemics through the kind of plants selected in a given area, time of planting, density of plant population and its management practices. Thus, the interaction of these components gives rise to disease **tetrahedron or disease pyramid.**



Simple interest and Compound interest disease

In simple interest disease, the pathogen is increases as a mathematical analogue to simple interest in money. It is also known as monocyclic disease as the pathogen has only one generation during the crop season. Primary inoculum is soil and seed borne. Initial inoculum plays an important role in spread of disease and rarely secondary inoculum. The rate of spread of disease is slow and the pathogen are mostly systemic in nature and do not produce propagules external to host during active season. Monocyclic diseases as exemplified by covered smut of barley (*Ustilago hordei*), grain smut of sorghum (*Sphacelotheca sorghi*), loose smut of wheat (*Ustilago segatum*), vascular wilt, post-harvest diseases and disease caused by soil-borne plant pathogens.

In compound interest disease, the pathogen is increases as a mathematical analogue to compound interest in money. It is also known as polycyclic disease as the pathogen has several or many generations during the crop season. Here, the pathogen produces spore rapidly and disseminated by air rapidly. The incubation period is short and sporulation occurs rapidly. Primary inoculum give rise to secondary inoculum and secondary infection play an important role in disease spread. In bimodal polycyclic diseases, different organs (blossoms or fruits) of the plant are attacked by pathogens at different time. Polycyclic diseases are exemplified by downy mildew, powdery mildew, rust and late blight of potato.

Slow epiphytotics and rapid epiphytotics

Slow epiphytotics occurs mostly in perennial population like trees. The pathogens are systemic and movement from one plant to another plant is slow. They have low death rate and the pathogen multiplies slowly due to lengthy incubation period or prolonged growth within the host tissue. Such epidemics are developed slowly (tardive). Environment influence is less. These type of epiphytotics is mostly resemble

with simple interest disease. Crop sanitation is the best management strategy for slow epidemics.

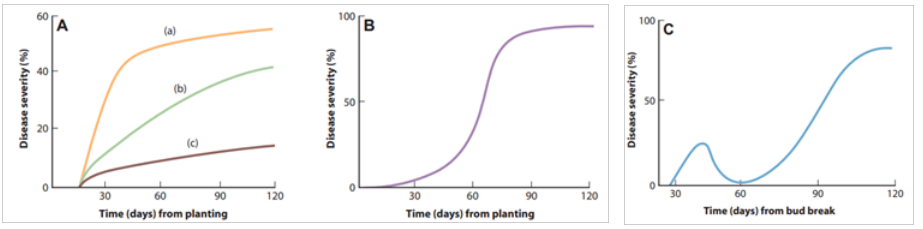
Rapid epiphytotics occurs mostly in annual crops. The pathogens are non-systemic, high birth rate, low death rate and have many generations within a short time. Environment influence is more. If graphically plotted, the rate of increase and decline, the epidemic rate curve will be bell shape or symmetrical as in late blight of potato or asymmetrical with the epidemic rate greater in the early season due to greater susceptibility of young leaves (apple scab and downy mildew) or asymmetrical with epidemic rate greater in the late season. Such epidemics are developed rapidly (explosive). These type of epiphytotics is mostly resemble with compound interest disease. Chemical control is the best management strategy for rapid epidemics.

Pattern and rate of epidemics

Patterns and rates are expressions of interactions of the structural components of epidemics as altered over time by environmental and human factors. **Disease-progress curve** shows the pattern of an epidemics progress over time, in terms of the amount of the disease tissues, numbers of lesions, or the numbers of diseased plants. The starting point and curve of a disease progression give information regarding the amount and time of appearance of the inoculum, changes in the host's susceptibility during the growth period, recurring meteorological occurrences, and the efficacy of preventative and cultural measures.

There are generally three types of Disease progress curve

a. **Saturation curve:** For monocyclic / Simple interest disease
b. **Sigmoid curve:** For polycyclic / Compound interest disease
c. **Bimodal curve:** Polycyclic disease affecting different plant organs



Disease gradient (dispersal) curve is a curve that depict the information of the spread of the pathogen in and out of the host population area. The severity and the number of the disease plants decrease steeply near the distance of the inoculum and less steeply at greater distance until it reaches zero or a low background level of the occasional disease plants.

Epidemic rate curve gives the amount of increase of disease per unit of time in the plant population under consideration and these curve is varied for different group of diseases.

Types of epidemic rate curve: -

1. **Symmetrical (bell shaped):** Late blight of potato
2. Asymmetrical rate curve:

» **Epidemic rate being greater early in the season:** Due to greater susceptibility in young leaves.

» Rust, Powdery mildew, Downy mildew and Apple scab.

» **Epidemic rate being greater late in the season:** Due to susceptibility of host increases at late season.

» *Alternaria* leaf blight and Vascular wilt.

Modelling of plant disease epidemics

Plant disease modelling helps us to predict and understand the direction as well as severity of the disease epidemics at future point of time. The ability to predict the direction and severity of the diseases help us to understand when and what type of management strategies can be used for its effective control. The model construct is generally simplified but roughly analogue to real epidemics for better picture and understanding of real scenario. For developing a plant disease model, database is developed containing information on the crop, the pathogen, the disease, the location of the weather station, and sensor (s) relative to the crop and the crop canopy. It also contains information on the input variables such as measured environmental variables, including precipitation, relative humidity, temperature and leaf wetness; calculated environmental variables such as dew point or degree hours; host variables such as inoculum potential, spore maturity and other pathogen factors. Thus, when we have enough information of these sub-components of an epidemic at different stages and under different conditions, a mathematical model has been developed, depicting the relationship between host, pathogen and environment and finally presented as an equation, as a table, as graph or as a simple statement.

Different model used for crop-yield loss estimation

1. **Critical point model**

» Estimates the loss for any given level of disease at a given time or any given time when a particular amount of disease is reached.

» Use linear regression, $\mathbf{Y = a + b\,x}$, where disease is taken as independent variable.

- » Applicable in late epidemic disease, fairly stable rate of infection and dry matter accumulation covered for only short period of time.
- » **Examples:** Rice blast, Wheat stem rust (first-second leaf unfold), Potato late blight, Potato cyst nematode.

2. Multiple point models

- » Estimates the disease at many points during the epidemic.
- » Use multiple regression, $\mathbf{Y = b_1X_1 + b_2X_2 + ... + b_nX_n}$, where Y is the percentage yield loss and a dependent variable and **X1, X2….Xn** are disease increment and independent variables.
- » Disease rating and regression coefficients is obtained as sum of the disease scored at different time, during disease progress and losses of crop yield.

3. Area under disease progress curve (AUPDC)

- » When disease severity or disease intensity is plotted against time, it gives disease progress curved and the area under this curved is called AUDPC.
- » It gives quantitative measure of disease severity $_{\text{with}}$ time.

$$\mathbf{A = \sum_{i=1}^{n} 1/2(S_i + S_i - 1) \times d}$$

Where,

- » $\mathbf{S_i}$ = Disease severity at end of week i
- » **K** = no. of successive evaluation of disease
- » **d** = interval between two evaluations

Computer simulation of epidemics

The computer is fitted with data containing various sub components of the epidemic and the control practices at specific points in time (such as weekly intervals). The computer then displays continuous information on the disease severity and spread of the disease over time and also the final crop and economic losses likely to occur during the disease epidemic. It allows to understand the importance of each subcomponent of an epidemic at the particular point in time, effect of each sub component on yield loss and help us to draw the effective management strategies in controlling the epidemic. The first computer simulation program, **EPIDEM** was designed against early blight of tomato and potato caused by *Alternaria solani* (1969). Some of the computer simulators are **EPIMAY** for Southern leaf blight of maize, **EPICORN** for Southern corn blight, **CERCOS** for Blight of celery,

MYCOS for Blight of Chrysanthemum, **EPIVEN** for Apple scab, **TOM-CAST** for early blight of potato, **PLASMO** for Downy mildew of grapes, **EPIVET** for Viral disease of potato, **EPIPIRE** for Cereal rust and aphids, **BLIGHT CAST, SIMCAST, NEGFRY** for Late blight of potato. Plant disease simulator which is more flexible is **EPIDEMIC** for stripe rust of wheat because we can have modified easily for other host-pathogen system also.

Forecasting of plant disease epidemics

Disease forecasting is a tool to predict the disease outbreak or intensity of a particular area in advance so that suitable control measures can be taken up to avoid crop losses. Forecasting helps us to determine whether, when and where a particular management strategy should be applied for its effective control. To develop a successful plant disease forecast, one must take into account several characteristics of the particular host, pathogen and environment. The Key to forecast any plant epidemic disease includes evaluation of epidemic thresholds, evaluation of economic damage threshold, assessment of initial inoculum and of disease and monitoring weather that affect in disease development.

Inoculum-based prediction

1. **Forecast based on amount of initial or primary inoculum:**
    - » Stewart's wilt of corn (*Erwinia stewartii*), Downy mildew or blue mold of tobacco (*Peronospora tabacina*), Pea root rot (*Aphanomyces euteiches*), Fire blight of apple and pear (*Erwinia amylovora*) and other disease caused by soil-borne pathogens such as *Sclerotium* and cyst nematodes
2. **Forecast based on amount of secondary inoculum:**
    - » Late blight of potato (*Phytophthora infestans*), Downy mildew of grapes (*Plasmopara viticola*), *Cercospora* leaf spot, Early blight of tomato (*Alternaria solani*) and *Septoria* leaf spot.
3. **Forecast based on amount of primary and secondary inoculum:**
    - » Apple scab, Black rot of grapes, *Botrytis* leaf blight and grey mold, cereal rusts, sugar beet yellows

Weather-based prediction

1. **Late blight of potato**
    - » When constant cool temperature between 10°C and 24°C prevails and relative humidity remains over 75% for at least 48 hrs or at least 90% for 10 hrs each day for 8 days then late blight infection will take place and 2

or 3 weeks later disease outbreak is excepted.

2. ***Cercospora* on peanuts and celery and *Exserohilum turcicum* on corn**
   - » It is predicted based on the temperature, the number of spores trap daily and the duration of the period with relative humidity near 100%. Infection period starts with relative humidity of 95- 100% for more than 10 hours.
3. **Bacterial blight of rice**
   - » Strong wind, rainy weather with temperature of 22 -26°C.
4. **Rice blast**
   - » Nycto- temperature of 20-24°C with relative humidity of 90% and above lasting for a week.

System approach in Epidemiology

For the majority of plant diseases, we need to come up with forecasting techniques that can take into account every significant factor of an epidemic, including the pathogen, the host, the weather, the time, and possibly the space. At the conceptual level, the first task of a system analyst is to identify the problem, its boundaries, and the objectives it seeks to achieve. Conceptual models are the first stage in model construction and try to logically arrange the information that is currently available or process thoughts for a critical analysis. The conceptual models display the order of processes and the level of decision-making. Additionally, it provides a comprehensive understanding of the system and the interrelated variables that affect the system's behaviour. The relationship between illness, weather, time, and space for wheat rusts was attempted to be correlated by Joshi et al. in 1974.

Joshi and Palmer (1973) used geophytopathology in field experiments to determine the causes of the non-functional nature of wheat stem rust over North-West India. At Karnal, Haryana, the spread of black, brown, and yellow rusts on wheat across time was monitored from a single point source. Their main goal was to show that the disease cannot spread, even in the unlikely event that stray stem rust urediniospores survive in the Himalayas. Meanwhile, North India's ongoing cold temperatures is helping to promote brown and yellow rusts on wheat. Stem rust spread is at its greatest between January and March, according to a disease gradient from a single point source. Therefore, a carefully thought out geophytopathological investigation can shed light on a lot of aspects that are now unclear.

Epidemiology of plant viruses

Over the past ten years, significant advancements have been made in our understanding

of plant virus infections, how they affect agriculture and wild plant ecosystems, and how ecological and evolutionary knowledge might be combined. More understanding has been given to the role and purpose of plant viruses in nature, for instance, the evolutionary paths that might lead from pathogenicity to mutualism and back again. However, more proof is needed before quantitative epidemiological research may be utilised to control diseases, describe, and explain short-term changes. Equally important is paying as much attention to the ecology of domesticated crops, farming practises, and their overall effects on plant viruses and pathogens as has been carried out for wild plant communities. Over the past few decades, epidemiological analysis techniques have also advanced. The effortless description of progression of diseases curves and disease gradients has been replaced by more sophisticated methods of epidemiological analysis over the past decade or so. When extensive empirical data on disease progression and related biotic and abiotic factors are available, modern computational and data mining techniques have transformed the ways in which theoretical epidemiological models can be evaluated and parameters estimated, for example, by linking Markov chain Monte Carlo (MCMC) methods to estimate the parameters of compartmental models of disease progress.

Instead than obtaining an adequate expression from theoretical models, there should be a larger effort made to compute the fundamental reproduction number from field data. A great deal of research has been done on characterising and, in some circumstances, forecasting the effects of a plant virus epidemic by characterising the spatial aggregation/structure in landscapes. In contrast to fungal plant diseases, more research is required to understand how climate change is affecting agricultural and wild populations of plant viruses, particularly where the impacts of virus infection vary in terms of host growth and aphid performance. There is a growing understanding that suitable spatial and temporal scales are necessary for forecasting when relating epidemiological research and analysis to disease management.

Crop variability is known to reduce the growth of arthropod pest populations and the harm they inflict; hence, effects on epidemics of plant virus vectors should also be considered. Despite being widely acknowledged to be effective in controlling arthropod pests, integrated pest management has not yet been properly applied to developing and putting into place integrated disease control strategies for plant viruses. Building on past empirical studies for process-driven model predictions of vector-related elements of virus epidemics, such as Plumb's infectivity index for BYDV, is a viable strategy. The increased importance and acknowledgement of the vector in all areas of virus epidemiology during the past ten years may be the most encouraging development. This is also a prevalent theme from the ecological and evolutionary viewpoints. Only after characterising the dynamic interaction

between the virus, vector, and host can it be decided how to best implement host resistance and tolerance in the field. The effects of releasing varieties with various resistance mechanisms can vary and have an impact on how well a variety is used. Deeper findings that such as how the interactions between horizontal and vertical transmission can have substantial epidemiological and evolutionary effects, have replaced the first basic categorization of the primary function of vector transmission in virus epidemiology. Expanding it to the field will be a difficulty because most of this understanding has been obtained from theoretical models and small laboratory/ microcosm trials.

The research on vector preference and the degree to which the virus is influencing the vector and host for its own purposes is a noteworthy aspect that highlights this necessity. Although much progress has been made in understanding the molecular underpinnings of vector preference, translating this knowledge into epidemiological and evolutionary implications remains a difficult task. It is now understood that co-infection with plant viruses is typical for many crops. How substantial is this co-infection, though, and how much of it is really the result of recent advancements in high throughput sequencing?

However, there needs to be more focus on how two or more viruses are interacting within the plant and much greater rigour in defining the outcomes of such interactions, whether antagonistic, neutral, or facilitative (use of the term synergistic requires considerable caution). There are instances where co-infection can cause more damage to a crop than do single viruses. Even if these interactions are defined, further research is still required to understand how they manifest in epidemics. In general, the role of the vector in sustaining coinfection in a plant population has not received enough attention in studies on how coinfection may or may not alter vector preference. Finally, even though multipartite viruses are rarely thought of as coinfections, the question of how multipartite viruses even survive in a plant population still remains for those that have genomic segments that are encapsidated separately and may require separate inoculations by a vector for a fully functional virus to be present in a plant cell.

Conclusion

Plant disease epidemics develop as a result of the timely combination of the same elements that result in plant disease: susceptible host plants, a virulent pathogen, and favorable environmental conditions over a relatively long period of time. To describe the interaction of the components of plant disease epidemics, the disease triangle, and describes the interaction of the components of plant disease, can be expanded to include time and humans. The effect of time on disease development

becomes apparent when one considers the importance of the time of year (i.e., the climatic conditions and stage of growth when host and pathogen may coexist), the duration and frequency of favorable temperature and rains, the time of appearance of the vector, the duration of the infection cycle of a particular disease, and so on. Humans affect the kind of plants grown in a given area, the degree of plant resistance, the numbers planted, time of planting, and density of the plants. The increase in the amount of disease at any one time is dependent of the initial amount of disease or initial inoculum, the rate of disease increase, and the duration of disease increase on the period of time involved. Control practices that reduce the amount of initial inoculum or initial plant disease cause delay of the specific point of the time at which a given disease level is reached but they do not change the rate of disease increase.

References

Agrios, G. N. (2005). Plant Disease Epidemiology. *Plant Pathology*, *5th Edition. Elsevier Academic Press, London, UK*, 266 - 289.

Alcalá-Briseño, R.I.; Casarrubias-Castillo, K.; López-Ley, D.; Garrett, K.A.; Silva-Rosales, L. Network analysis of the papaya orchard virome from two agroecological regions of Chiapas, Mexico. mSystems 2020,5, e00423-19

Alexander, H.M.; Mauck, K.E.; Whitfield, A.E.; Garrett, K.A.; Malmstrom, C.M. Plant-virus interactions and the agro-ecological interface. Eur. J. Plant Pathol. 2014, 138, 529–547. [CrossRef]

Allen-Perkins, A.; Estrada, E. Mathematical modeling for sustainable aphid control in agriculture via intercropping. Proc. R. Soc. A 2019, 475, a20190136. [CrossRef] [PubMed]

Batuman, O.; Turini, T.A.; LeStrange, M.; Stoddard, S.; Miyao, G.; Aegerter, B.J.; Chen, L.-F.; McRoberts, N.; Ullman, D.E.; Gilbertson, R.L. Development of an IPM strategy for thrips and Tomato spotted wilt virus in processing tomatoes in the Central Valley of California. Pathogens 2020, 9, 636. [CrossRef]

Beltran-Beltran, A.K.; Santillán-Galicia, M.T.; Guzmán-Franco, A.W.; Teliz-Ortiz, D.; Gutiérrez-Espinoza, M.A.; Romero-Rosales, F.; Robles-García, P.L. Incidence of Citrus leprosis virus C and Orchid fleck dichorhavirus citrus strain in mites of the genus Brevipalpus in Mexico. J. Econ. Entomol. 2020, 113, 1576–1581. [CrossRef]

Burdon, J.J.; Thrall, P.H. What have we learned from studies of wild plant-pathogen associations?—The dynamic interplay of time, space and life-history. Eur. J. Plant Pathol. 2014, 138, 417–429. [CrossRef]

Chen, Y.H.; Gols, R.; Benrey, B. Crop domestication and its impact on naturally selected trophic interactions. Annu. Rev. Entomol. 2015, 60, 35–58. [CrossRef] [PubMed]

Fargette, D.; Vié, K. Simulation of the effects of host resistance, reversion, and cutting selection on incidence of African cassava mosaic virus and yield losses in cassava. Phytopathology 1995, 85, 370–375. [CrossRef]

Hamelin, F.M.; Allen, L.J.S.; Bokil, V.A.; Gross, L.J.; Hilker, F.M.; Jeger, M.J.; Manore, C.A.; Power, A.G.; Rúa, M.A.; Cunniffe, N.J. Co-infections by non-interacting pathogens are not independent: Deriving new tests of interaction. PLoS Biol. 2019, 17, e3000551. [CrossRef] [PubMed]

Holt, J.; Chancellor, T.C.B. Modelling the spatio-temporal deployment of resistant varieties to reduce the incidence of rice tungro disease in a dynamic cropping system. Plant Pathol. 1999, 48, 453–461. [CrossRef]

Jeger, M.J. Bottlenecks in IPM. Crop Prot. 2000, 19, 787–792. [CrossRef]

Kendig, A.E.; Borer, E.T.; Mitchell, C.E.; Power, A.G.; Seabloom, E.W. Characteristics and drivers of plant virus community spatial patterns in US west coast grasslands. Oikos 2017, 126, 1281–1290. [CrossRef]

MacDiarmid, R.; Rodoni, B.; Melcher, U.; Ochoa-Corona, F.; Roossinck, M. Biosecurity implications of new technology and discovery in plant virus research. PLoS Pathog. 2013, 9, e1003337. [CrossRef] [PubMed]

McLeish, M.J.; Sacristán, S.; Fraile, A.; García-Arenal, F. Co-infection organises epidemiological networks of viruses and hosts and reveals hubs of transmission. Phytopathology 2019, 109, 1003–1010. [CrossRef] [PubMed]

Mehrotra, R. S. & Aggarwal, A. Plant Disease Epidemiology and Plant Disease Forecasting. *Plant Pathology. 3rd Edition. McGraw Hill Education (India) Pvt. Ltd, New Delhi*, 247- 265.

Naidu, R.A.; Maree, H.J.; Burger, J.T. Grapevine leafroll disease and associated viruses: A unique pathosystem. Annu. Rev. Phytopathol. 2015, 53, 613–634. [CrossRef] [PubMed]

Osada, Y.; Yamakita, T.; Shoda-Kagaya, E.; Liebhold, A.M.; Yamanaka, T. Disentangling the drivers of invasion spread in a vector-borne tree disease. J. Anim. Ecol. 2018, 8, 1512–1524. [CrossRef] [PubMed]

Reitz, S.R.; Gao, Y.; Kirk, W.D.J.; Hoddle, M.S.; Leiss, K.A.; Funderburk, J.E. Invasion biology, ecology, and management of Western Flower Thrips. Annu. Rev. Entomol. 2020, 65, 17–37. [CrossRef] [PubMed]

Roberts, M.G.; Heesterbeek, J.A.P. Model-consistent estimation of the basic reproduction number from the incidence of an emerging infection. J. Math. Biol. 2007, 55, 803–816. [CrossRef]

Singh, R. S. (2018). Epidemiology. *Introduction to Principles of Plant Pathology, 5th Edition. Scientific International (Pvt.) Ltd, New Delhi,* 117- 132.

Untiveros, M.; Fuentes, S.; Salazar, L.F. Synergistic interaction of Sweet potato chlorotic stunt virus (Crinivirus) with carla-, cucumo-, ipomo-, and potyviruses infecting sweet potato. Plant Dis. 2007, 91, 669–676. [CrossRef] [PubMed]

Van den Driessche, P. Reproduction numbers of infectious disease models. Inf. Dis. Model. 2017, 2, 288–303.[CrossRef]

Varghese, A.; Drovandi, C.; Mira, A.; Mengersen, K. Estimating a novel stochastic model for within-field disease dynamics of banana bunchy top virus via approximate Bayesian computation. PLoS Comput. Biol. 2020, 16, e1007878. [CrossRef]

Wilhoit, L.R. Modelling the population dynamics of different aphid genotypes in plant variety mixtures. Ecol. Model. 1991, 55, 257–283. [CrossRef]

5 Enzymes and Toxins in Pathogenesis

Saru Sara Sam[1], Alby John[2] and Deepa R. Chandran[3]

[1,2,3]Department of Plant Pathology, College of Agriculture, Vellayani, Kerala Agricultural University, Thiruvananthapuram- 695522, Kerala

Abstract

Pathogenesis implies the series of development of an infection and the continuous events which leads to the development of a disease because of a chain of events occurring at the morphology and metabolism of a plant tissue which is been attacked by a pathogen, chemical or physical agent. The events in disease cycle includes host recognition, infection, invasion, colonization, growth and reproduction of pathogen, symptom development, dissemination of pathogen, incubation, attachment, penetration and finally the disease development. The important determinants for the initiation of disease and its severity depends on the amount of toxin and enzymes produced. Moreover, the plant produces various components as a defense mechanism against the invading pathogens. The toxins and enzymes produced by the pathogens can counteract the defense produced and leads to the plant-pathogen interactions. Numerous plant diseases release enzymes such cellulases, pectinases, and ligninases to break down parts of the plant cell wall such that the pathogens can utilize the photosynthates for growth and development. The toxins produced by the pathogens can interfere in plants metabolism and produces various symptoms specific to the pathogen. Thus, the combinations of toxin and enzymes are the major determinants for disease initiation and spread in plant hosts.

Keywords : Pathogenesis, Toxin, Enzymes, plant-pathogen interactions

Introduction

Pathogenesis implies the series of development of an infection and the continuous events which leads to the development of a disease because of a chain of events occurring at the morphology and metabolism of a plant tissue which is been attacked by a pathogen, chemical or physical agent. The pathogenesis in an infection cycle implies the various mechanism through which the etiological factors stimulate the disease development. Pathogenesis can be synonymously used for describing the development changes of a disease, which can be acute, chronic or recurrent. Pathogenesis originates from a Greek word pathos which means "disease" and the word genesis which means "creation". There are different chemical components secreted by the pathogens which are essential as they bring out the disease developmental activities (Vidhyasekaran, 2007). The above mentioned components include enzymes, toxins, plant growth regulators and infectious polysaccharides.

Enzymes and toxins impart the most determinants in plant the events of pathogenesis, through the process by which infectious plant pathogens, which includes fungi, bacteria, viruses, and nematodes can cause diseases developments in plants. Pathogens such as fungi, nematodes and bacteria are capable to produce more than one of the toxins and enzymes in the interactions imparted in pathogen-host. Viruses and viroids hardly secrete enzymes but a few of them could encapsidate the enzymes in a particle which they produce. Plant pathogenic organisms are able to continually secrete enzymes in a constitutive manner or at least when the organism comes in contact with the plant. The upcoming pathogens are developing and developed numerous strategies in invading and colonizing the plant parts where toxins and enzymes are being used as the important determinants to overcome the defense imparted by plants thus ultimately helps in the establishment of infections (Pfeilmeier *et al.,* 2016). The major roles of enzymes and toxins in plant pathogenesis are utilization of enzymes in cell wall degradation, toxins imparting damage to host tissues, toxins required in the interference of host defense mechanisms, and various enzymes for photosynthates acquisition and the detoxification of plant defense.

Numerous plant diseases release enzymes such cellulases, pectinases, and ligninases to break down parts of the plant cell wall, which is what is meant by the term "enzymes for plant cell wall degradation." Invading cells and gaining access to nutrients is made possible by these enzymes' breakdown of structural polysaccharides like cellulose, hemicellulose, and pectin. The pathogens create toxins that can directly harm plant tissues, whereas the toxins produced by bacteria can start host tissue damage (Sexton *et al.,* 2006). These toxins break cell membranes, cause programmed cell death (apoptosis), impair protein synthesis, and interfere with a variety of cellular functions. Toxins provide an environment that is conducive to pathogen colonization

and nutrient uptake by producing tissue necrosis or wilting. The chemicals created for controlling host defense mechanisms by secreting compounds that control plant defense mechanisms. These poisons can undermine or circumvent plant defenses, which enables the pathogen to spread infection (Brader *et al.,* 2017). By targeting and suppressing plant defense signaling pathways, for instance, pathogen effector proteins can help the pathogen evade detection and colonization.

For the purpose of improving nutrient uptake, microorganisms develop enzymes that break down plant tissues and macromolecules like proteins, carbohydrates, and lipids into smaller molecules that are simple for them to absorb and use as nutrients (Khan et al., 2011). These enzymes hydrolyze proteins, lipids, and starch, respectively, and comprise proteases, lipases, and amylases. The substances that plants produce as a defense can be detoxified by pathogens. As a result, bacteria create detoxifying enzymes such detoxifying oxidases or hydrolases that neutralize or modify the harmful substances, enabling the pathogen to survive and spread disease (Kimura, 2001).

In general, the pathogenic factors that allow plant pathogens get past plant defenses mechanisms, infiltrate plant tissues, gain nutrition, and trick the host immune system are the enzymes and poisons they create. For the purpose of creating ways to prevent plant diseases and safeguard crop plants, it is critical to comprehend these mechanisms.

Enzymes in Pathogenesis

Plant pathogens mostly engage in chemical-based activities. Numerous enzymes secreted by phytopathogens weaken the host's defensive coats. Thus, phytopathogens use enzymes as chemical weapons. Through stomata or other naturally occurring holes, pathogens can enter the host and initiate pathogenesis. The mode of entry includes lenticels, hydathodes, and nectarthodes, wounds, or physically breaking through the host's defensive layers by means of various strategies.

Fungal pathogens can enter through one or more of the methods mentioned above. Due to the fact that the majority of bacteria do not secrete enzymes that degrade cell walls, they can enter the host tissues through any gap in the host surface. Large protein molecules typically make up enzymes, which catalyze biological reactions. Some enzymes are created only when they are needed, while others are constitutive, active all the time, and catalyze all the vital reactions needed by the organism (Doehlemann *et al.,* 2017).

Pathogens typically make their first communication with their host near the surface of plants, such as leaves for pathogens that fly. To enter the host, they must first compromise the host's defenses. Multiple layers of protection are applied to plants to

prevent easy invasion by any creature. Cuticle, which is made up of cutin and waxes combined together, is the outermost and most protective covering of leaves. Pectin and hemicelluloses are located close together. These make up the middle lamella. The most prevalent polymer in plants, cellulose, makes up the cell wall.

The internal cell walls, which are made of cellulose, pectins, hemicelluloses, and structural proteins, as well as the middle lamella, which is formed of hemicelluloses, are broken down, allowing pathogens to enter parenchymatous tissues more easily. The pathogenic organism frequently secretes ligases, cellulases, cutinases, and cellulolytic enzymes. By allowing viruses to infect and colonize host plants, enzymes play a crucial part in the development of plant diseases. Different enzymes produced by plant pathogens help with nutrition acquisition, host defense suppression, and penetration of plant tissues.

The cuticle and plant cell wall are the first surfaces an organism comes into contact with for pathogenesis. The complex wax called cutin, which makes up the cuticle, impregnates the cellulose wall. The structural component of the cell wall is cellulose, which is also made up of the matrix components hemicellulose, glycoproteins, pectin, and lignin.

Therefore, one or more enzymes that break down these chemical compounds are responsible for their entry into live parenchymatous tissues and the breakdown of the middle lamella.

The cuticle layer's cutin is broken down by cutinases, which presoftens the tissue for mechanical penetration or as the initial stage of tissue breakdown. The major cell wall's middle lamella is made up of pectic materials, which also create an amorphous gel between the cellulose microfibrils. Pectin methyl esterases (PME), polygalacturonases (PG), and pectin lyases or transeliminases are pectin-degrading compounds that are frequently referred to as pectinases or pectolytic enzymes.

Small groups like methyl groups (CH3) are frequently removed by pectin methyl esterases, which alters solubility and slows down the pace at which polygalacturonase and pectin lyase break chains. While pectin lyases cleave chains by removing a water molecule from the bond, polygalacturonases do the opposite. Many different plant diseases, especially the soft rot diseases, are caused by pectin-degrading enzymes (Tucker *et al.,* 2001).

Cellulase

Cellulose is converted to glucose by cellulase enzymes. Three enzymes are included in cellulase, including 1,4-endoglucanase, cellobiohydrolase, and glucosidase. Bacteria and fungi create cellulases. Endoglucanases randomly hydrolyze internal glycosidic

links, which causes the length of the polymer to rapidly decrease and the concentration of reducing sugar to gradually rise.

By removing cellobiose from either the reducing or nonreducing ends of cellulose chains, exoglucanases hydrolyze the chains, causing a rapid release of reducing sugars but little change in polymer length. Cellobiose is created when endoglucanases and exoglucanases work in concert to break down cellulose into glucose.

While Claviceps purpurea early infection phase on Secale cereale expresses cellobiohydrolase, which increases the pathogen's virulence, Macrophomina phaseolina uses an endocellulase for pathogenicity. One endocellulase is produced by the phytopathogenic Alternaria alternata, which plays a significant role in the progression of disease in persimmon fruit. The development of symptoms was inhibited by Xanthomonas oryzae pv. oryzae mutant strains lacking cellulase, which cause bacterial leaf blight in rice (Annis et al., 1997).

During the pathogenesis process, Erwinia carotovora subsp. carotovora can generate a significant amount of cellulase, which weakens the host cell wall and promotes faster infection rates and longer disease duration. This weakening of the cell wall was greatly diminished in potato tissues of Erwinia carotovora subsp. carotovora cellulase-inhibited mutants. The hydrolysis of lignocellulosic polymer materials occurs naturally as a result of synergistic interactions among cellulolytic microbes.

Hemicellulases

Complex polysaccharide polymers called hemicelluloses connect the terminals of pectic compounds to cellulose microfibrils. Numerous hemicellulases have been discovered in numerous plant pathogenic fungi due to the wide variety of hemicelluloses, which include xyloglucans, glucomannans, glactomannans, arabinoglucans, etc. The middle lamella, which is rich in pectin, and the chemically intricate cell wall, which secretes a wide range of enzymes that break down the middle lamella and cell wall, are the physical barriers that Fusarium encounters at this stage.

When *Fusaria* sp. and other fungal plant diseases attack cereals, pectin-degrading enzymes are typically the first middle lamella- and cell wall-degrading enzymes released. Their activity then exposes other cell wall polysaccharides for degradation by hemicellulases and cellulases. Plant defense proteins called polygalacturonase-inhibiting proteins (PGIPs) decrease the hydrolytic activity of endoPGs and encourage the buildup of long-chain oligogalacturonides (OGs), which trigger a range of defensive reactions. The leucine reach repeat (LRR) protein superfamily, which also includes the byproducts of various plant resistance genes, includes PGIPs as a member (Ma *et al.,* 2015).

Ligninases

The most common type of white-rot fungi mineralize the components of cell walls (cellulose, hemicelluloses, and lignins) and extensively breakdown lignins, giving rotting wood a bleached appearance. Heme-peroxidases and laccases released by fungi that utilise the oxidants H_2O_2 and O_2 as electron acceptors participate in the breakdown of lignin. Lignin peroxidases, manganese peroxidases, and versatile peroxidases are the three primary heme-peroxidases. Lignin is able to oxidize non-phenolic lignin units, as can numerous peroxidases.

In the middle lamella and secondary cell wall of plants, lignin, a phenylpropanoid, can be detected. The stiff, woody character of woody tissues is mostly conferred by lignin. Wood rotting fungus are a rare breed, with about 500 species. Basidiomycetes, or "white-rot" fungus, are responsible for the majority of lignin breakdown. The ligninases that these fungus create allow them to use lignin (Asemoloye *et al.,* 2021).

Numerous fungi species have been found to have laccase activity, and the enzyme has already been isolated from dozens of different species. This could suggest that laccases are extracellular enzymes that are typically found in the majority of fungal species. Environmentally speaking, the wood-rotting basidiomycetes that produce laccase are closely connected to the wood-degrading ascomycetes that include the soft rotter Trichoderma and the ligninolytic *Bothryosphaeria*. A variety of phenolic compounds are used as hydrogen donors by laccase as it catalyzes the conversion of O_2 to H_2O.

Toxins in Pathogenesis

The pathogenesis of plant infections is greatly impacted by toxins. Pathogens generate these poisons to aid in the spread of colonisation and disease. Plant toxins are the compounds needed for virulence and aggressivity, respectively. Pathogenicity, which is a qualitative term, refers to a pathogen's capacity to cause disease (Logrieco *et al.,* 2011). Virulence is a quantitative term that refers to the quantity or extent of the disease caused. Interest in the toxin theory of plant disease was rekindled by the identification of the host-specific toxin victorin. Toxins can harm or even kill the host when they interact with living host cells. Depending on the concentration, toxins can produce either conventional or unusual symptoms (Yamane *et al.,* 2010).

Toxins are frequently categorized as generic, host-specific, or selective. Nonspecific toxins can affect both animals and plants in general, while host-specific toxins are highly active exclusively against the host of the disease that produces them. Because of their great potency, host-specific toxins are active against even disease-resistant plants at low doses and have an extremely narrow target range for susceptible plants. Toxins decrease host defense mechanisms, aid in the colonization and establishment

of pathogens within plant tissues, and hasten the spread of illness. For the purpose of managing and controlling plant diseases, it is essential to comprehend how toxins are produced and how they affect plant hosts (Durbin, 2012).

Moreover, if the toxin is host-specific and targets a certain species or cultivar of plant. These toxins aid in the pathogen's host specificity, enabling it to infect and spread illness only to particular plant hosts while sparing others. For instance, *Cochliobolus victoriae* host-specific toxin causes Victoria blight in oats but has no effect on other plant species. While pathogens that draw their nutrition from dead or dying host cells create necrotrophic toxins, which cause necrosis or cell death in plant tissues. These toxins degrade host cells, liberate nutrients, and foster an environment that is conducive to the pathogen's expansion and colonization (Daub, 2005).

For instance, the *Fusicoccum amygdali* produced toxin fusicoccin results in necrosis in stone fruit trees. Some microorganisms create toxins that prevent the synthesis of chlorophyll or photosynthesis in plant cells, which ultimately leads to chlorosis or the yellowing of plant tissues, which weakens the plant's overall health and diminishes its capacity to perform photosynthesis (Upadhyay *et al.,* 2015). Coronatine, a toxin produced by *Xanthomonas campestris* pv. *vesicatoria*, causes chlorosis in tomato and pepper plants. A few diseases create toxins that imitate or interfere with plant hormones, preventing normal plant development and growth (Scheffer, 1991).

The toxins affect plant physiology in favor of the pathogen by manipulating plant hormone signaling pathways. A toxin termed gibberellin, which is produced by *Gibberella fujikuroi* and mimics the plant hormone gibberellic acid, promotes aberrant growth in rice plants. Pathogens that directly harm plant tissues and cells produce phytotoxins. These toxins cause cell death and tissue damage by rupturing cellular membranes, impeding protein synthesis, interfering with DNA replication, or inducing oxidative stress. For instance, the phytotoxin syringomycin, which is made by the bacterium *Pseudomonas syringae*, disrupts plant cell membranes and results in leaf spot symptoms (Walton, 1996).

Non-Specific Toxins

Regardless of whether they are a host or non-host of the pathogen causing the toxin, many plants can be harmed by it. Nonspecific toxins have a broad spectrum of effects on plants regardless of whether they are a host or non-host of the pathogen producing them, in contrast to host-specific toxins, which target a specific species or cultivar of plant. The infections that create these poisons often affect a variety of plant species and have a wide host range (Horbach *et al.*, 2011). Non-specific toxins frequently function as generic virulence factors that increase the generating pathogen's overall pathogenicity.

Non-specific toxins can operate in a number of ways, such as by rupturing cell membranes, impairing cellular functions, reducing host defenses, or even causing cell death. These poisons allow the virus to travel both inside and between various plant species, establish infections, and take advantage of host resources. As they aid in the onset and progression of certain pathogen-caused diseases, nonspecific toxins play a crucial role in plant pathology (Sharma *et al.,* 2019).

Tab toxin

The "wildfire disease of tobacco" is a result of tab toxin. Leaves with this condition have necrotic patches with a yellow halo around them. Culture filtrates of pathogen or its pure toxins that produce symptoms that are equivalent to those of a tobacco wildfire may cause the sickness. Necrotic patches with a yellow halo are produced on leaves by strains that produce toxins. The toxin is not host-specific because similar effects can be seen on a variety of hosts. Chemically, tabtoxin is a dipeptide made up of the amino acids tabtoxinine and threonine. The active toxin, tabtoxinine, is released when tabtoxin is broken inside the cell.

The common amino acid threonine with the hitherto unidentified amino acid tabtoxinine make up the dipeptide known as tabtoxin. Although tabtoxin itself is not poisonous, in the cell it undergoes hydrolysis and releases tabtoxinine, the poison that actually causes harm. Because tabtoxin, through tabtoxinine, deactivates the enzyme glutamine synthetase, glutamine levels are lowered, hazardous ammonia concentrations build up, and tabtoxin is poisonous to cells as a result. The latter separates photosynthesis and photorespiration and breaks down the chloroplast's thylakoid membrane, leading to chlorosis and ultimately necrosis. The toxin's effects diminish the plant's capacity to actively react to the bacterium (Does *et al.,* 2007).

Phaseolotoxin

P. syringae pv. phaseolicola and actinidiae, which cause bacterial canker on kiwifruit and halo blight on legumes, both produce phaseolotoxin. toxin connected to the "halo blight" bacterial bean blight. The toxin itself has the ability to cause symptoms of the sickness that the bacterium causes. The toxin is an ornithine-alanine-arginine tripeptide with a phosphosulfinyl group. Phaseolotoxin is a tripeptide made of ornithine, alanine, and homoarginine that is connected to a sulfodiaminophosphinyl moiety. The poisonous constituent of the toxin, phosphosulfinylornithine, is released by enzymatic cleavage within the cells. The inactivation of the enzyme ornithine carbamoyltransferase has cellular effects (Bronson, 1991).

P. syringae pv. phaseolicola and actinidiae produce phaseolotoxin, which causes bacterial canker on kiwifruit and halo blight on legumes. toxin responsible for

the "halo blight"—one of the bacterial bean blights. The toxin itself is capable of causing the disease that the bacterium causes to manifest. The toxin is a tripeptide made up of ornithine, alanine, and arginine that has a phosphosulfinyl group. A sulfodiaminophosphinyl moiety connected to a tripeptide made of ornithine, alanine, and homoarginine makes up the structure of phaseolotoxin (Annis *et al,* 1997). The poisonous moiety, phosphosulfinylornithine, is released by enzymatic cleavage of the toxin inside of cells. The inhibition of the enzyme ornithine carbamoyltransferase has an impact on the cellular structure.

Both prokaryotes and eukaryotes depend on the urea cycle, and *P. syringae pv. phaseolicola* produces two isozymes of OCTase to counteract phaseolotoxin's harmful effects. One isozyme is susceptible (SOCTase) and the other is resistant to the toxin (ROCTase). *P. syringae pv. phaseolicola* produces the ROCTase isoform when the conditions are favorable for the formation of phaseolotoxin.

Tentoxin

A naturally occurring cyclic tetrapeptide called tentoxin is made by the phytopathogenic fungus *Alternaria alternata.* Chlorosis is selectively induced in a number of germination seedling plants. As a result, tentoxin has the potential to be employed as a natural herbicide. *Alternaria alternata* became the first source of tentoxin. This organism primarily causes seedling diseases in a wide variety of plant types. When more than one-third of the leaf surface is chlorotic, a seedling will die, and vigor will be reduced with less chlorosis.

Recent studies have also employed tentoxin to stop the polyphenol oxidase (PPO) activity in seedlings of higher plants. A chloroplast-coupling factor protein that is involved in energy transfer as well as the suppression of light-dependent phosphorylation of ADP to produce ATP is targeted by the toxin, which is a cyclic tripeptide (King *et al.,* 2011). Tentoxin has a reversible chlorotic effect, and it prevents decotyledonized embryos from utilizing the nutrients in the growth medium and making up for the absence of cotyledons. The citrus system provides a special opportunity to investigate the connection between tentoxin's impact on chloroplast ATPase activity and the production of chlorosis.

Host Specific Toxins

Host-specific toxins (HSTs) are pathogen effectors that only affect the host species and only in genotypes of that host that express a particular, frequently dominant susceptibility gene. They cause toxicity and promote disease in the host species. These are metabolic byproducts from pathogenic microorganisms that are only hazardous to their hosts. *Helminthosporium victoriae, Periconia circinata*, and *Alternaria kikuchiana*

all have significant host-specific toxins. These compounds are very poisonous to susceptible organisms yet almost non-hazardous to other living creatures.

All of the symptoms of the diseases brought on by the corresponding infections are produced by the recognized host-specific toxins. The appealing theory that poisons contribute to plant illnesses has been around since deBary's time. When the toxin theory is reduced to its simplest form, it may be said that all of the symptoms of a certain disease are caused by a toxic byproduct of the bacterium that causes that disease. When the toxin is artificially delivered at amounts that could be reasonably anticipated in or around the diseased plant, it causes all of the disease's symptoms in a susceptible host. The ability of the pathogen to create the toxin directly correlates with its capacity to cause disease, and both the pathogen and the toxin demonstrate similar host specificity. Additionally, just one toxin is implicated.

The first toxin to be discovered that met the aforementioned requirements was made by *Cochliobolus (Helminthosporium) victoriae*. With the development and widespread use of the oat variety Victoria' and its derivatives, which had the Vb gene for resistance to the crown rust disease, Helminthosporium leaf blight of oats first occurred in 1945. The fungus that causes the victoria lines enters plant roots and, while it does so, produces a poison that spreads to the leaves, where it kills the plant and causes severe leaf blight. All other oat kinds and plant species are either resistant to the poison or their sensitivity to it is inversely correlated with how susceptible they are to the fungus (Trail *et al.,* 1990).

In addition to creating internal biochemical and histochemical changes that are comparable to those caused by the disease, the toxin also causes outward symptoms that are similar to those caused by the pathogen. The toxin is a sophisticated chlorinated cyclic pentapeptide chemically. Studies on the built environment show that the plasma membrane is the toxin's main target. Here, it appears to attach to proteins and, through an as-yet-unidentified process, affects the metabolism of cells that are sensitive.

T toxin

The fungus *Cochliobolus heterostrophus*, formerly known as *Helminthosporium maidis,* causes the common corn disease known as "Southern Corn Leaf Blight" that produces the toxin. Race T of the fungus, which first arrived in the United States in 1968, is responsible for producing T-toxin. By 1970, corn with Texas male-sterile (TMS) cytoplasm experienced significant losses all across the corn belt. It was discovered that corn cultivars with normal cytoplasm were resistant to both the fungus and the toxin. Chemically speaking, the toxin is a combination of long, linear polyketols with 35–45 carbon atoms (Horbach *et al.,* 2011).

The toxin only affects susceptible cells' mitochondria, where ATP generation is suppressed. The T-urf13 gene, which is expressed in the inner membrane of mitochondria, produces a 13 kDa protein with 115 amino acids as its target in plants that are vulnerable to it. The uncoupling/dissipation of membrane energization, mitochondrial enlargement, leaking of mitochondrial NAD+, and ensuing suppression of respiration are all caused by the T toxin binding to this protein (Manici *et al.,* 1997).

Hc-Toxin

The HC-Toxin, which causes maize leaf spot disease, is made by the fungus *Cochliobolus carbonum (Helminthosporium).* Only selected maize (corn) lines have the toxin. The maize pathogen's host-selective toxin, HC toxin, prevented maize histone deacetylase from working. By interfering with reversible histone acetylation, which is thought to be involved in the regulation of essential cellular processes such chromatin structure, cell cycle progression, and gene expression, HC toxin aids in the establishment of pathogenic compatibility between *C. carbonum* and maize.

Conclusion

Pathogenesis is the key determinant for disease development. The events in disease cycle includes host recognition, infection, invasion, colonization, growth and reproduction of pathogen, symptom development, dissemination of pathogen, incubation, attachment, penetration and finally the disease development. For the events to occur toxins and enzymes plays the major role in development. The pathogens developed numerous strategies in invading and colonizing the plant parts where toxins and enzymes are being used as the important determinants to overcome the defense imparted by plants thus ultimately helps in the establishment of infections.

The major roles of enzymes and toxins in plant pathogenesis are utilization of enzymes in cell wall degradation, toxins imparting damage to host tissues, toxins required in the interference of host defense mechanisms, and various enzymes for photosynthates acquisition and the detoxification of plant defense. Toxins and enzymes have both quantitative and qualitative aspects. The severity of disease development depends on these components. The diverse spectrum of their potential uses is illustrated by the fact that the poisonous proteins produced by plants can have either positive or negative impacts on people and animals. By producing and storing a variety of chemical and protein-based poisonous chemicals, plants have evolved a sophisticated defensive system to recognize and react to invasive species.

There are several secondary plant substances that have been identified, including ribosome-inactivating proteins, lectins, plant protease inhibitors, -amylase inhibitors, canatoxin-like proteins, ureases, arcelin, antimicrobial peptides, and pore-forming toxins.

Due to their biological properties, these substances have been used by humans in a wide range of sectors, including the production of drugs, natural herbicides, therapeutic/pharmaceutical agents, and genetic applications in agriculture and medicine.

References

Annis, S. L., & Goodwin, P. H. (1997). Recent advances in the molecular genetics of plant cell wall-degrading enzymes produced by plant pathogenic fungi. *European Journal of Plant Pathology*, *103*, 1-14.

Asemoloye, M. D., Marchisio, M. A., Gupta, V. K., & Pecoraro, L. (2021). Genome-based engineering of ligninolytic enzymes in fungi. *Microbial cell factories*, *20*, 1-18.

Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L. J., & Sessitsch, A. (2017). Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. *Annual Review of Phytopathology*, *55*, 61-83.

Bronson, C. R. (1991). The genetics of phytotoxin production by plant pathogenic fungi. *Experientia*, *47*, 771-776.

Daub, M. E., Herrero, S., & Chung, K. R. (2005). Photoactivated perylenequinone toxins in fungal pathogenesis of plants. *FEMS Microbiology Letters*, *252*(2), 197-206.

Doehlemann, G., Ökmen, B., Zhu, W., & Sharon, A. (2017). Plant pathogenic fungi. *Microbiology spectrum*, *5*(1), 5-1.

Durbin, R. (Ed.). (2012). *Toxins in plant disease*. Elsevier.

Horbach, R., Navarro-Quesada, A. R., Knogge, W., & Deising, H. B. (2011). When and how to kill a plant cell: infection strategies of plant pathogenic fungi. *Journal of plant physiology*, *168*(1), 51-62.

Horbach, R., Navarro-Quesada, A. R., Knogge, W., & Deising, H. B. (2011). When and how to kill a plant cell: infection strategies of plant pathogenic fungi. *Journal of plant physiology*, *168*(1), 51-62.

Khan, T. A., Mazid, M., & Mohammad, F. (2011). Role of ascorbic acid against pathogenesis in plants. *Journal of Stress Physiology & Biochemistry*, *7*(3), 222-234.

Kimura, M., Anzai, H., & Yamaguchi, I. (2001). Microbial toxins in plant-pathogen interactions: Biosynthesis, resistance mechanisms, and significance. *The Journal of general and applied microbiology*, *47*(4), 149-160.

King, B. C., Waxman, K. D., Nenni, N. V., Walker, L. P., Bergstrom, G. C., & Gibson, D. M. (2011). Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. *Biotechnology for biofuels*, *4*(1), 1-14.

Logrieco, A., Moretti, A., & Solfrizzo, M. (2009). Alternaria toxins and plant diseases:

an overview of origin, occurrence and risks. *World Mycotoxin Journal*, *2*(2), 129-140.

Ma, Y., Han, C., Chen, J., Li, H., He, K., Liu, A., & Li, D. (2015). Fungal cellulase is an elicitor but its enzymatic activity is not required for its elicitor activity. *Molecular plant pathology*, *16*(1), 14-26.

Manici, L. M., Lazzeri, L., & Palmieri, S. (1997). In vitro fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. *Journal of agricultural and food chemistry*, *45*(7), 2768-2773.

Pfeilmeier, S., Caly, D. L., & Malone, J. G. (2016). Bacterial pathogenesis of plants: future challenges from a microbial perspective: challenges in bacterial molecular plant pathology. *Molecular plant pathology*, *17*(8), 1298-1313.

Scheffer, R. P. (1991). Role of toxins in evolution and ecology of plant pathogenic fungi. *Experientia*, *47*, 804-811.

Sexton, A. C., & Howlett, B. J. (2006). Parallels in fungal pathogenesis on plant and animal hosts. *Eukaryotic cell*, *5*(12), 1941-1949.

Sharma, N., & Gautam, A. K. (2019). Early pathogenicity events in plant pathogenic fungi: A comprehensive review. In *Biol. Forum* (Vol. 11, No. 1, pp. 24-34).

Trail, F., & Köller, W. (1990). Diversity of cutinases from plant pathogenic fungi: Evidence for a relationship between enzyme properties and tissue specificity. *Physiological and molecular plant pathology*, *36*(6), 495-508.

Tucker, S. L., & Talbot, N. J. (2001). Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annual review of phytopathology*, *39*(1), 385-417.

Upadhyay, A., Mooyottu, S., Yin, H., Surendran Nair, M., Bhattaram, V., & Venkitanarayanan, K. (2015). Inhibiting microbial toxins using plant-derived compounds and plant extracts. *Medicines*, *2*(3), 186-211.

van der Does, H. C., & Rep, M. (2007). Virulence genes and the evolution of host specificity in plant-pathogenic fungi. *Molecular Plant-Microbe Interactions*, *20*(10), 1175-1182.

Vidhyasekaran, P. (2007). *Fungal pathogenesis in plants and crops: molecular biology and host defense mechanisms*. CRC Press.

Walton, J. D. (1996). Host-selective toxins: agents of compatibility. *The plant cell*, *8*(10), 1723.

Yamane, H., Konno, K., Sabelis, M., Takabayashi, J., Sassa, T., & Oikawa, H. (2010). Chemical defence and toxins of plants.

6 Infection Process During Fungal Pathogen Interaction in Plants

Deepa R. Chandran, Saru Sara Sam and Alby John

P.h.D Scholars, Department of Plant Pathology, College of Agriculture, Vellayani, Kerala Agricultural University, Thiruvananthapuram - 695522, Kerala

Abstract

In this chapter, the typical pathogenic processes exhibited by fungi is discussed. The pathogenesis process of fungi is very intricate. In a nutshell, it involves the dispersal and arrival of an infectious particle in the vicinity of the host, adhesion to the host, recognition of the host which may happen even before the adhesion or attachment, penetration into the host, invasive growth within the host followed by lesion or symptom development in the host, and finally production of additional infectious particles for further dispersal and spread. This process is more or less cyclic. Digging deep into the infection process can pave way to development of novel strategies for managing many fungal diseases of crop plants that are even affecting the global economy.

Keywords: Fungi, pathogen, infection process, defence, colonisation

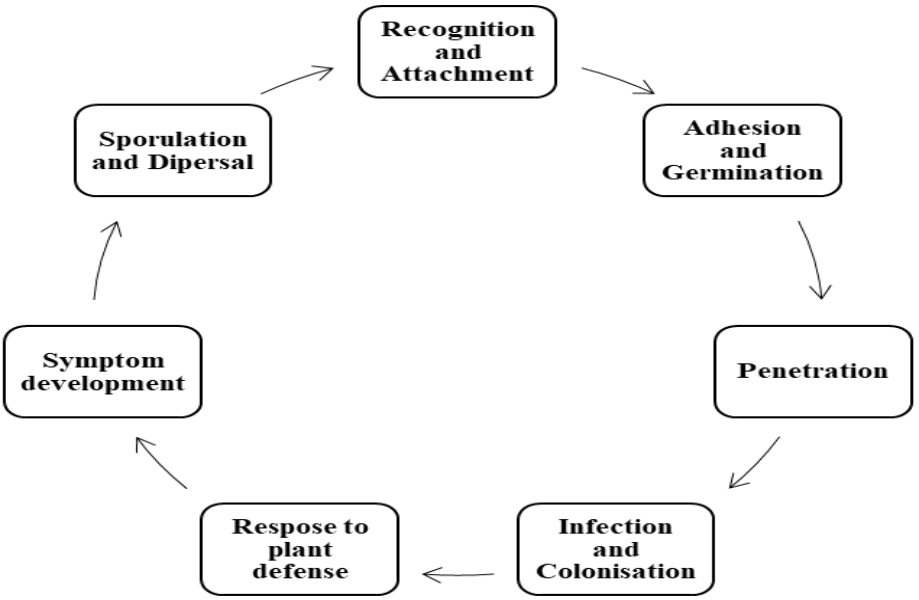
Introduction

Fungi is a group of eukaryotic carbon heterotrophic animals with a wide range of adaptations. They have successfully inhabited most of the natural settings. The bulk of fungus are stringent saprophytes, and only a part now recognized are able to colonize plants, and even fewer of them may spread disease. Phytopathogenic fungi are the primary cause of infectious diseases that affect crop plants, but they also play a significant role in the less dramatic but still significant yield losses that have made fungal pathogens a significant economic concern and drawn the attention of both farmers as well as scientists.

It must be noted that the plants are constantly and permanently exposed to a wide variety of possible pathogens. The development of disease is only possible if there is presence of a simultaneous combination of a susceptible host, a virulent pathogen, and a conducive environment. In other words, not every pathogen can infect every plant, and not every plant is susceptible to all the phytopathogens. In actuality, only a very small percentage of these pathogens are able to successfully invade each plant host and spread illness.

In addition, the pathogen population and host plant undergo developmental changes throughout their life cycles, which alter pathogen virulence and host vulnerability. According to Montesinos et al. (2002), environmental factors including temperature, water availability, and plant surface wetness have a significant impact on disease. For all of these factors, disease development is typically less common than one might anticipate. Moreover, plants have developed complex and wide range of defense mechanisms throughout the course of their interaction with the pathogens. Proteins and other chemical substances created by both organisms serve as the main weapons in a conflict when a pathogen, such as a fungus, tries to infect a plant. Plants create an incredible variety of defensive chemicals, and new ones are continually being found every day.

For a fungal pathogen to cause a disease in a plant, it has to undergo multiple stages of infection during the fungal-pathogen interactions. Here is an overall summary of the process:



Recognition and Attachment

The interaction between a fungal pathogen and a plant starts with a kind of communication between them. If the plant is a host, then it will recognise the pathogen and it will not happen in case of non-host plants. This recognition is aided with the help of some sensors/receptors present in the plant surface. These sensors will sense the incoming signals of pathogen which can be chemical or physical/thigmotropic. The recognition can trigger the onset of a cascade of biochemical reactions within the plant as well as the pathogen.

Before causing disease, host as well as pathogen must overcome several challenges. A pathogen is typically a non-host pathogen if it is unable to overcome the initial barriers. The same hurdles pose substantial difficulties for host pathogens as well and sharply lower their success rates. Any normal plant-pathogen interaction begins with the pathogen recognizing the host and then being perceived by the host's defensive mechanisms. To drive cell differentiation and produce crucial pathogenic genes, pathogens need particular signals from the plant surface (Thordal-Christensen, 2003). The timely perception of the pathogen by the plant is essential for the activation of defense mechanisms.

Moreover, through air currents, soil, or vectors like insects, the fungus' spores or hyphae typically come into touch with the plant's surface. The spores adhere to the plant's surface after identifying chemical signals from the surface.

Adhesion and Germination

Spores adhere to the host by both physical and chemical processes. The fungal spores develop into structures like appressoria or germ tubes that aid in their adhesion to the surface of the plant. Specialized organs called appressoria emit enzymes or apply mechanical pressure to aid in penetration.

According to Mendgen et al. (1996), the host's hydrophobicity, surface hardness, plant surface components, and topographical characteristics are the primary signals. For instance, in the case of Phytophthora pathogens, both host-specific factors like isoflavones and non-host-specific factors like amino acids, calcium, and electrical fields influence zoospore taxis, encystment, cyst germination, and hyphal chemotropism, which are significant in directing the pathogen to potential infection sites (Tyler, 2002).

Some plant pathogens can detect many kinds of anticlinal walls or stomatal apertures, thanks to thigmotropic responses that direct germ tubes on leaf surfaces. While the composition of the surface wax is crucial for the establishment of a differentiated appressorium in barely powdery mildew fungus, hyphal differentiation

of rust fungus is driven by the topography of the plant's surface (Hoch and Staples, 1991).

Another illustration is given by *Colletotrichum gloesporioides* and *C. musae*, the rot-causing pathogens in climacteric fruits like tomato and banana (*Musa* spp.), respectively. These organisms use the signal provided by the fruit-ripening hormone ethylene for germination and differentiation (Flaishman and Kolattukudy, 1994). When these fungi's conidia are exposed to micromolar amounts of ethylene, the conidia begin to germinate and produce many appressoria on hard surfaces. Exogenous ethylene had to be added for transgenic tomato fruits incapable of producing ethylene to enable fungal germination and apressorium development.

Broad bean, the host of the rust fungus *Uromyces fabae*, releases volatile chemicals in response. In tests conducted *in vitro* without the presence of the host, the fungus detects these volatiles. On artificial membranes, the growth of haustoria is encouraged by three of them - nonanal, decanal, and hexenyl acetate (Mendgen et al., 2006).

One fungus may react differently to signals sent by the same plant surface depending on its stage of life (*Mendgen et al., 1996*). Thus, for instance, the dikaryotic stage of *Uromyces appendiculatus* differentiates appressoria in response to the proper dimensions of a ridge formed by the stomatal lips of the guard cell, whereas the monokaryotic stage of this organism differentiates infection structures in response to its hardness (Freytag and Mendgen, 1991).

In order for the fungal structures to adhere passively or non-metabolically to host surfaces, hydrophobic interactions between spores, hyphae, and the cuticle are frequently present. To help hyphae sense the plant surface and distinguish between distinct infection structures, a second stage involves the production of a protein, glycoprotein, or carbohydrate coating surrounding the germ tube and portions of the cuticle (Mendgen et al., 1996). According to some spores' production of cutinases or esterases, the cuticle is eroded and aids in fungal adherence (Deising et al., 1992).

Penetration

Next step following adherence is penetration. By physically breaking through the cell wall or by using enzymatic processes to break down the cell wall constituents, the fungal hyphae enter the plant's epidermal cells. The fungus can infiltrate the plant tissues after making successful penetration. Likewise, the transition from extracellular to invasive growth is also marked by penetration through the cuticle and the plant cell wall, which is a critical and essential step in the pathogenesis of most biotrophic fungi (Panstruga and Schulze-Lefert, 2003).

Amazingly diverse invasive techniques have been developed by fungi. They

have developed methods to infiltrate plant tissue, enhance plant development, and reproduce in order to colonize plants. Natural openings or wounds are a common entry point for bacteria, viruses, and some opportunistic fungal parasites. Contrarily, many real phytopathogenic fungi have developed means of actively navigating the cuticle and epidermal cell wall, the plant's outer structural barrier. The penetration of the cuticle and plant cell wall is supported by targeted and controlled secretion of cutinases and a variety of cell wall-degrading enzymes, production of phytotoxic metabolites like mycotoxins and oxalic acid, and/or an increase in pressure within the fungal infection structures for causing physical punctures (Punja, 2001).

Fungi typically release a mixture of hydrolytic enzymes, such as cutinases, cellulases, pectin lyases, pectate lyase, polygalacturonase, and proteases, to gain entry. These enzymes are also necessary for their saprophytic lifestyle. Additionally, not all hydrolytic enzymes may be strictly necessary for penetration. However, this does not rule out tailoring their structure or biosynthetic regulation to the unique requirements of a pathogen on a particular host plant. Cutinase is thought to be a critical role in the penetration process, and it has been proposed that enzymatic breakdown of cutin, the structural polymer of the plant cuticle, is essential for fungal pathogenicity. It may also play a role in the prepenetration process, for example, by modifying the cuticle's adhesive properties to make it easier for fungi to attach to plant surfaces, or by releasing signal molecules necessary for the early development of fungi on plants (Kolattukudy et al., 1995).

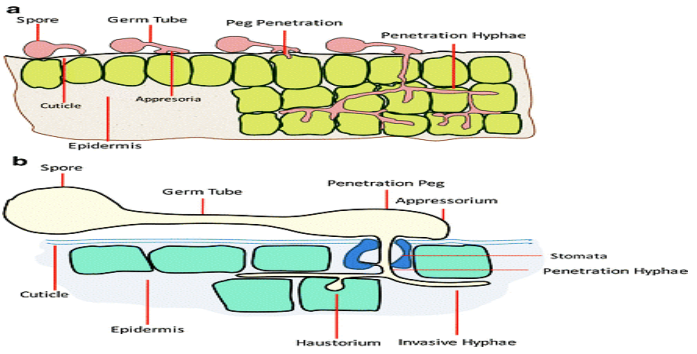


Fig A: Spore germination and entry via direct penetration (source: Sharma and Jangid, 2017)

Some fungi, as an alternative to or in addition to hydrolytic enzymes, have created a more intricate and sophisticated method to pierce the cuticle of host plants (Fig A.). Appressoria, which are specialized penetration organs that are formed by phytopathogenic fungi at the tip of their germ tubes, are firmly adhered

to the plant surface by extracellular adhesives. Melanin incorporation significantly reduces the porosity of the appressorium wall of mechanically penetrating fungi as it grows, allowing considerable turgor pressure to accumulate inside. A small region at the base of the appressorium that is kept free of wall material and melanin is where this pressure is most effectively concentrated. An infection peg grows from this penetration pore and penetrates the cuticle and cell wall, perhaps with the aid of hydrolytic enzymes. Studies on the *Magnaporthe grisea*, the rice blast fungus, have demonstrated the significance of melanin for infection peg penetration (Kubo and Furusawa, 1991). Additionally, according to Howard et al. (1991), melanized appressoria of *M. grisea* are capable of forcing penetration pegs through polymeric membranes.

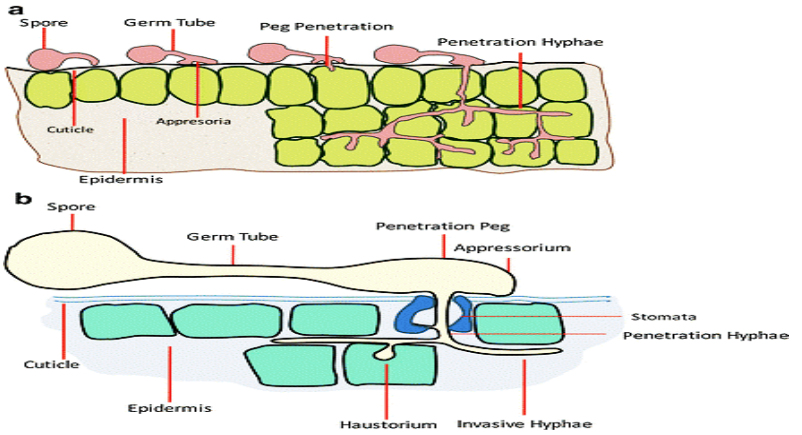


Fig B: Spore germination and entry into host via stomata (source: Sharma and Jangid, 2017)

Other fungal species, such as certain rusts, lack a direct penetration method and instead enter through the stomata, avoiding the plant cuticle and outer cell wall (Fig B.). To find these perforations on the plant surface, these fungi have evolved a poorly known mechanism.

Therefore, it is possible that a variety of factors will work together to control penetration. These elements may also include plant surface features, fungal spore germination and germ tube activators or inhibitors, as well as fungus-specific chemicals.

Infection and Colonization

The fungal hyphae continue to develop and spread once they have entered the plant, either by entering the plant cells directly or through the intercellular gaps. The development of either haustoria or intracellular hyphae is a characteristic of the obligatory biotrophic fungus and some of the hemibiotrophic fungi. Following

penetration of the cell wall, these structures form inside the plant cell and are encircled by an invaginated plant plasma membrane. Haustoria are determinate branches of intercellular, intracellular, or epicuticular hyphae that terminate within a host cell (in the case of powdery mildews). Contrarily, many intracellular hyphae can move from cell to cell and still resemble haustoria in both function and structure (Fig B.). Haustoria and intracellular hyphae have been linked to signaling, communication, and avoiding recognition by the host in addition to acquiring water and nutrients from the host.

To obtain nutrients and resources, the fungus secretes enzymes and toxins that aid in destroying plant cell walls. These toxins and enzymes degrade the plant cells and release monomers that can serve as the ready to use carbon source for the fungal pathogens. The release of toxins or substances that resemble plant hormones, which alter the physiology of the plant to the pathogen's advantage, is frequently the following step in a fungal attempt to colonize a plant species after penetration. This interference can simply include destroying plant cells to prevent them from absorbing nutrients or it can involve a more subtle rerouting of the cellular machinery (Keen, 1986). It is frequently accomplished by the development of phytotoxins that are more or less specific to particular plants. While some toxins are active in a variety of plant species, others are host-selective. Toxins are the factors that determine specificity in some plant-fungus interactions, according to genetic and biochemical research. In these circumstances, sensitivity to the toxins or resistance to the fungus always correspond with one another. This has led to a lot of interest in these host-selective toxins, which are mostly generated by species of the fungi Alternaria and Cochliobolus. Host-nonselective toxins, on the other hand, affect both hosts and nonhost organisms.

These toxins may be extremely important throughout the progression of fungal disease on a specific host, despite the fact that their non-selectivity conflicts with a role in determining host range. They could also be seen as relics of earlier fungal evolutions toward phytopathogenicity, whose activity in most plants may be suppressed by detoxification or other processes.

Plant Defense Response

Plants are constantly exposed to a wide range of pathogens. However, only a few number of these pathogens can actually infect each type of plant with illness. On the other hand, only a small number of plants are susceptible to each pathogen's illness. By definition, all other plants are non-host plants, and the invading organisms are non-host pathogens. A plant is said to be resistant to a disease if it is unable to attack it, which indicates that the plant is resistant to the pathogen and it cannot live on the plant.

Once the pathogen gains entry, the plant starts developing a defense as soon as it notices the infection. This may entail the induction of defense genes, the creation of antimicrobial substances, the fortification of cell walls, and the attraction of immune cells to the site of the infection. Fungal pathogens are likely to be recognized by plants quickly after coming into contact with them and will be met with an active defensive mechanism. Therefore, it is obvious that an effective surveillance system is essential for early danger detection and the subsequent activation of defense-specific enzymes that operate to halt future fungal development. Successful pathogens must then counteract the plant's defense mechanism, and so on. The highly developed plant-fungus interactions are evidence of such coevolutionary dynamics.

Early pathogen detection by the plant is essential for the induction of defense responses. The term elicitor is currently frequently used to describe any molecule that sets off a plant defensive response, while being originally intended to describe any substance capable of stimulating the formation of phytoalexins. Molecules of pathogen origin (exogenous elicitors) or host components released or altered by pathogen effectors (endogenous elicitors) are also included in this expanded definition of an elicitor (Nurnberger, 1999). Examples of elicitors that have been isolated from the necrotrophic fungus Botrytis cinerea include botrycin and cinerein.

Two main categories of elicitors – general/non-specific, and specific/race-specific elicitors—can be distinguished based on mode of action. It appears that the activation of the first line of plant defenses is mediated by general elicitors, which comprise proteins, glycoproteins, peptides, polysaccharides, and lipids. They are emitted during attacks by both host and non-host pathogens, and they alert both host and non-host plants to the probable presence of pathogens in a non-cultivar-specific way (Nurnberger, 1999; Thordal-Christensen, 2003). General elicitors are clearly essential for the rejection of non-host pathogens, as is becoming increasingly clear. Due to receptor-mediated sensing, they invariably reveal the invader to the plant's defense system and are frequently constitutive and important for the pathogen lifetime (Nurnberger et al., 2004). When the pathogen is entering the host, general elicitors are released through the enzymatic breakdown of pathogen cell wall polymers. Such elicitors, such as chitin and glucan oligomers, are frequently necessary to the microbe and are hypothesized to be used by plants during recognition. However, given that some of them are only recognized by a small number of plants, the non-specific nature of generic elicitors is relative (Shibuya and Minami, 2001).

Race-specific elicitors are virulence effectors or avirulence factors that are unique and specific for a certain pathogen and display restricted specificity, frequently inducing reactions in a cultivar-specific manner. Specific elicitors, as opposed to general elicitors, are expressed by avr genes and cause cultivar-specific responses,

which are frequently accompanied with hypersensitive response. According to the gene-for-gene concept, this particular recognition is controlled by the direct or indirect interaction of host R proteins with relevant pathogen-derived avr effectors (Caldo et al., 2004). When the complementary protein equivalents of the R-gene are able to recognize the avr factors, they function as specialized elicitors of plant defense rather than virulence or pathogenic factors. For example, tomato plants expressing the *Cf-9* resistance gene will experience HR when exposed to the *Cf avr9* produced by the tomato leaf mold, *Cladosporium fulvum* (Van der Hoorn et al., 2002; Lauge et al., 2000).

Symptom Development

The manifestation of disease symptoms may depend on the particular interaction between the plant and the fungus infection. Symptoms caused by fungi might be widespread or limited. Most frequently, fungal infections result in general necrosis of the host tissue and frequently cause stunting, deformities, and aberrant alterations in the tissue and organs of plants. These symptoms may include wilting, necrosis, chlorosis, lesions, aberrant growth, and a general deterioration in plant health. In most situations, lesion formation is the ultimate outcome of infection.

The physical appearance of pathogen, sign, is one of the most recognizable and easily distinguishable traits of a fungal infection. For the accurate identification and diagnosis of a disease, look for hyphae, mycelia, fruiting bodies, and spores of the fungus pathogen. Fungi produce fruiting structures that range in size from microscopic to macroscopic. They have distinctive qualities and are available in a variety of shapes and arrangements. In most situations, the disease can be accurately identified using the fruiting bodies, spores, and mycelium (signs).

The following symptoms are common in fungal infections whether alone or in combination with other fungal pathogens:

Leaf spots caused by fungi frequently appear as isolated lesions with collapsed and necrotic tissue. Leaf spots typically have a dry texture, but they are not papery-dry. E.g brown leaf spot of rice (*Cochliobolus miyabeanus*)

Anthracnose is a lesion that resembles an ulcer and may be necrotic and sunken. These lesions can develop on the host's fruit, flowers, and stems, for example., anthracnose of pulses caused by *Colletotrichum* spp.

Rusts: Infected plants frequently develop several small lesions on stems or leaves. These lesions are typically rust-coloured; however they can also be black or white. E.g., wheat stem rust

Smuts are cluster of spores or mycelium that appear on seeds as galls or as seeds

that have been replaced by spores as in heads smut of barley

Often sunken, a *canker* is a localized necrotic lesion on woody tissue, such as European Canker in apple (*Nectria galligena*),

Damping off is the term for a seedling's sudden collapse and death. Either the seed rots before it sprouts, or the seedling rots at the soil line, collapses, and perishes. This illness is brought on by a number of soil-born fungi including *Pythium*, *Rhizoctonia*, and *Fusarium*.

Dieback refers to the progressive death of shoots and twigs, which typically begins at the affected plant part's tip. E.g. Cherry and Apple dieback (*Monilinia sp*.).

Soft and dry *root rots* cause the rot and decomposition of fleshy leaves, roots, tubers, and fruit. e.g: Phytophthora Root Rot (*P. cinnamomi*) and *Armaillaria spp.* Tree Root Rot.

Blight is characterized by the rapid, widespread browning and death of leaves, floral components, stems, and branches. Examples are potato late blight (*Phytophthora infestans*) and tomato early blight (*Alternaria tomatophilai*, previously *A. solani*).

Wilts are loss of overall turgidity in the vascular tissue. E.g., Verticillium wilt of cowpea.

Mildews Powdery and downy mildews

Scab is a small, localized lesion on the host plant's leaves, fruits, tubers, and other plant parts. Scab causes the host's skin to become rough and crusty. Example include Apple Scab caused by *Venturia inaequalis*.

Leaf curls are curled, thickened, and distorted leaves formed after the fungal infections in some plants, for example, Peach leaf curl caused by *Taphrina sp*.

Galls are swollen portions of plant organs that are typically brought on by excessive plant cell growth or multiplication, such as the clubroot of crucifers (*Plasmodiophora brassicae*).

Sporulation and Dispersal

The pathogen will colonise the plant and at some points of unfavourable conditions they start to convert their vegetative cells to reproductive structures or directly spores. Although direct infection by hyphae is possible, the most typical way for new infections to start is by spore dispersal. Spores can be of different types sexual or asexual, endospores or exospores etc. According to their motile nature, spores can be divided into two categories, motile and non-motile spores. Non-motile spores include sexual spores such as oospores, ascospores, rust urediniospores, sclerotia,

and conidiospores and asexual spores like sporangiospores, and conidia. Motile spores include zoospores, or mobile spores with flagella, which are common among oomycetes and chytrid fungi.

These spores that a fungal pathogen develops can be spread by a variety of agents, including wind, water, insects, and human activity. These spores allow the disease to spread to other host plants and keep the infection cycle going. The spores land on appropriate substrate (host plant part) following the dispersal and repeat the infection cycle.

Conclusion

It is vital to remember that different fungal infections and plant species can have diverse infection processes. The effectiveness of the interaction between the pathogen's virulence and the plant's defense responses depends on the balance of defense mechanisms that plants have evolved to combat fungal infections. Some fungi complete only one infection cycle in host whereas certain others complete multiple infection cycles within the same host plant. Based on the pathogen, host as well as the environment the pattern and form of the infection process may vary. All these have to be taken into account while formulating management strategies for warding off the fungal diseases in plants.

References

Caldo, R.A., Nettleton, D., and Wise, R. P. (2004). Interaction-dependent gene expression in Mla-specified response to barley powdery mildew. Plant Cell 16: 2514–2528.

Deising, H., Nicholson, R. L., Haug, M., Howard, R. J., and Mendgen, K. (1992). Adhesion pad formation and the involvement of cutinase and esterases in the attachment of uredospores to the host cuticle. Plant Cell 4: 1101– 1111.

Flaishman, M. A., and Kolattukudy, P. E. (1994). Timing of fungal invasion using host's ripening hormone as a signal. Proc. Natl. Acad. Sci. USA 91: 6579– 6583.

Freytag, S., and Mendgen, K. (1991). Carbohydrates on the surface of urediniospore- and basidiospore-derived infection structures of heteroecious and autoecious rust fungi. New Phytologist 119: 527–534.

Hoch, H., and Staples, R. (1991). Signaling for infection structure formation in fungi. In: The Fungal Spore and Disease Initiation in Plants and Animals. 25. Hoch, H., Ed., Plenum Press, New York.

Keen, N.T. (1986). Pathogenic strategies of fungi. In Recognition in Microbe-Plant Symbiotic and Pathogenic Interactions, B. Lugtenberg, ed (Berlin: Springer-Verlag). pp. 171-188.

Kolattukudy, P.E., Rogers, L.M., Li, D., Hwang, C.S., and Flaishman, M.A. (1995). Surface signaling in pathogenesis. Proc. Natl. Acad. Sci. USA 92, 4080-4087.

Kubo, Y., and Furusawa, I. (1991). Melanin biosynthesis. Prerequisite for successful invasion of the host by appressoria of Colletotrichum and Pyricularia. In: The Fungal Spore and Disease Initiation in Plants and Animals. 205. Hoch, H., Ed., Plenum, New York.

Lauge, R., Goodwin, P. H., de Wit, P. J., and Joosten, M. H. (2000). Specific HR-associated recognition of secreted proteins from Cladosporium fulvum occurs in both host and non-host plants. Plant J. 23: 735–745.

Mendgen, K., Hahn, M., and Deising, H. (1996). Morphogenesis and mechanisms of penetration by plant pathogenic fungi. Annu. Rev. Phytopathol. 34: 367– 386.

Mendgen, K., Wirsel, S. G., Jux, A., Hoffmann, J., and Boland, W. (2006). Volatiles modulate the development of plant pathogenic rust fungi. Planta In press.

Montesinos, E., Bonaterra, A., Badosa, E., Franc´es, J., Alemany, J., Llorente, I., and Moragrega, C. (2002). Plant-microbe interactions and the new biotechnological methods of plant disease control. Int. Microbiol. 5: 169–175.

N¨urnberger, T. (1999). Signal perception in plant pathogen defense. Cell. Mol. Life Sci. (CMLS) 55: 167–182.

Nurnberger, T., Brunner, F., Kemmerling, B., and Piater, L. (2004). Innate immunity in plants and animals: striking similarities and obvious differences. Immunol. Rev. 198: 249–266.

Panstruga, R., and Schulze-Lefert, P. (2003). Corruption of host seven transmembrane proteins by pathogenic microbes: a common theme in animals and plants? Microbes Infect. 5: 429–437.

Punja, Z. (2001). Genetic engineering of plants to enhance resistance to fungal pathogen - a review of progress and future prospects. Can. J. Plant Pathol. 23: 216–235.

Repka, V. (2006). Early defence responses induced by two distinct elicitors derived from a Botrytis cinerea in grapevine leaves and cell suspensions. Biol. Plant., 50: 94–106.

Sharma, R., and Jangid, K. (2017). Role of Quorum Sensing in Fungal Morphogenesis and athogenesis. In: Mérillon, JM., Ramawat, K. (eds) Fungal Metabolites. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-319-25001-4_38

Shibuya, N., and Minami, E. (2001). Oligosaccharide signalling for defence responses in plant. Physiol. Mol. Plant Pathol. 59: 223–233.

Thordal-Christensen, H. (2003). Fresh insights into processes of nonhost resistance. Curr. Opin. Plant Biol. 6: 351–357.

Tyler, B. M. (2002). Molecular basis of recognition between phytophthora pathogens and their hosts. Annu. Rev. Phytopathol. 40: 137–167.

Van der Hoorn, R. A., DeWit, P. J., and Joosten, M. H. (2002). Balancing selection favors guarding resistance proteins. Trends Plant Sci. 7: 67–71.

7 Pathogen Defense Mechanism in Plants

H. A. Shekhada[1], C. M. Bhaliya[2], J. J. Padsala[3] and R. L. Joshi[4]

[1]Ph.D. Scholar, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, (Junagadh) 362 001
[2]Assistant Research Scientist, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, (Junagadh) 362 001
[3]Ph.D. Scholar, Navsari Agricultural University, (Navsari) 369 450
[4]Ph.D. Scholar, Navsari Agricultural University, (Navsari) 369 450

Abstract

Higher plant's ability to withstand stress is vital to their existence. As consequently, plants have evolved to adapt to stress through modifications to their normal patterns of defense mechanism. In response to a pathogen attack, every plant has a wide spectrum of defense mechanisms, including first and foremost structural defense mechanisms and later cellular defense mechanisms as a result of host-pathogen interactions. These mechanisms include the production of waxes, small and thick cuticle, natural opening structures, cork layer, abscission layer, Tyloses formation, gum deposition, hyphal sheathing, hypersensitive response (HR), antimicrobial substance present and release by plant, secondary metabolites such as phytoalexins, PR proteins and others. The role of the structural and biochemical defense mechanisms of plants against pathogen such as fungi, bacteria, viruses, nematodes, and others has been demonstrated and described in this chapter.

Keyword: Structural and biochemical defense mechanisms

Introduction

Plant lacking an immune system comparable to animals. Each type of pathogen, such as fungi, bacteria, viruses, mycoplasma, nematodes, etc., has the capacity to harm

a plant. Crop yields may be drastically decreased by certain infections. However, because of their defense mechanisms, many plants do survive despite being attacked by these pathogens. Plants respond to pathogen attack by erecting a highly coordinated series of molecular, cellular and tissue-based defense barriers. All plants have the capacity to activate these defenses. However, if they are activated too little, too late, or in the wrong place, they will fail to restrict the pathogen and the plant will be susceptible. Pathogens respond by escaping or suppressing plant defense responses or by rendering these responses impotent, for example by detoxifying plant antibiotics.

Plants employ a combination of defense mechanisms known as host resistance against pathogens, which include (1) structural defense mechanism that act as physical barriers and prevent the pathogen from entering and spreading through the plant; and (2) biochemical defense mechanism occurring in the plant's cells and tissues and produce substances that are either toxic to the pathogen or that create conditions that inhibit the pathogen from establishing in the plant. Different host-pathogen systems are made up of structural characteristics and metabolic reactions that are used for the defense of plants. But despite the type of defense or resistance a host plant uses to ward off a disease or an abiotic agent, it is ultimately determined by the genetic makeup of both the host plant and the pathogen (genes), either directly or indirectly (Tom Schultz, 2007).

Structural defense mechanisms

The surface of the host is first line of defense against the pathogen. The pathogen must adhere to the surface and penetrate, if it is to cause infection. Structural defense mechanism is mainly two type: 1. Pre-existing structural defense mechanism (Wax, Thick cuticle, Thickness and toughness of the outer wall of epidermal cells, Stomata, Sclerenchyma cells and Lenticel) and 2. Post-infectional or induced structural defense mechanism (Cellular defense structure- hyphal sheathing and Histological defense structure- formation of cork layer, formation of abscission layer, formation of tyloses and deposition of gums). Saprophytes lack the ability to penetrate these natural barriers.

Pre-existing structural defense mechanism

Cuticular wax: Wax-mixtures of long chain aliphatic compounds get deposited on the cuticular surface of some plants. Deposition of wax on the cuticular surface is thought to play a defensive role by It is synthesized by epidermis and extremely hydrophobic. As a result, the pathogen does not get sufficient water to germinate or multiply. In addition, a negative charge usually develops on the leaf surface due to the presence of fatty acids – the main component of cuticle. The negative charge

prevents/reduces the chance of infection by many pathogens. It forms a protective coating on plant leaves and fruit. Fungi which degrade the wax is *Puccinia hordei*. Generally wax layer is not degraded by the pathogen, thicker the wax layer more is the resistance.

Cuticle and Epidermal cell: Plant structures like tough and thick epidermal cells play a role in defense against pathogens, these are important factors in the resistance of some plants to certain pathogens by making direct penetration fungal pathogens difficult or impossible. Ex: Disease resistance in *Barberry* species infected with *Puccinia graminis tritici* has been attributed to the tough outer epidermal cells with a thick cuticle. Similarly, the ability of *Taphrina deformans* to infect only young, newly unfolded leaves has been attributed to the inability of germ tubes to penetrate the thicker cuticles of older leaves. In linseed, cuticle acts as a barrier against *Melampsora lini*.

The wall having same thickness, but differing in toughness due to the presence or absence of lignin and silicic acid, which prevent the further spread of pathogen. Ex: Silicification and lignification of epidermal cells offers protection against *Pyricularia oryzae* and *Streptomyces scabies* in paddy and potato, respectively. The activity of this type of cutinolytic enzyme in isolates of *Fusarium solani* and *Fusarium oxysporum* f. sp. *pisi* is directly related to their aggressiveness on pea stems, indicating that pathogens unable to dissolve the cuticle at the point of penetration are exclude (David and John, 1997).

Sclerenchyma cells: The presence of secondary cell walls in sclerenchyma, xylem or older plant tissue often retards pathogen development, Brittle cells help in mechanical support of the plant. Example, to angular leaf spots where pathogen spread is restricted by leaf veins.

Structure of natural opening

Stomata: Most of pathogen enters plants through natural openings. *Puccinia graminis* f.sp. *tritici* wheat enters the host only when the stomata are open. Uredospores germinate early in the morning and stomata of wheat crop are also remain open in the morning at 6 am that enables entry of germ tube of uredospore. Hope cultivar of wheat is resistant to *Puccinia graminis* f.sp. *tritici* because the stomata in Hope cultivar are opens at 9 am in the morning, during that time the germ tube of germinated in the morning will dries up (Tom Schultz, 2007). Structure of stomata provides resistance to penetration by certain pathogenic bacteria. Ex: Citrus variety, Szinkum, is resistant to citrus canker because the smaller size of stomatal opening.

The black pod pathogen, *Phytophthora palmivora*, enters cocoa pods through stomata. Cocoa genotypes that produce pods with few and relatively smaller stomata,

allow fewer lesions to establish than genotypes with more numerous, larger stomata. Not surprisingly, as the pathogen enters through stomatal pores, there is no correlation between cuticle thickness or pod case hardness and resistance to black pod.

Lenticels: Lenticels are openings on fruit, stem and tubers that are filled with loosely connected cells that allow the passage of air. Shape and internal structure of lenticels can increase or decreases the incidence of fruit diseases. Ex. Small and suberized lenticels will resist potato scab pathogen, *Streptomyces scabies*.

Nectaries: It provides openings in the epidermis and may play a defensive role due to high osmotic concentration of the nectar. In resistant varieties of apple, presence of abundant hairs in the nectaries acts as a defense mechanism while susceptible varieties are devoid of abundant hairs.

Hydathodes: Black rot of cabbage is internally seed borne disease, it enters through the hydathodes.

Post-infectional/Induced structural defense mechanism

Most of the pathogens manage to penetrate their hosts through wounds and natural opening and to produce various degree of infection. Pathogen penetration through the host surface induced the structural defense mechanism in the host cells. These may be regarded as: Histological defense barriers (cork layer, abscission layers and tyloses formation) and cellular defense structures (hyphal sheathing).

Histological defense mechanism

Cork layers formation: Infection by fungi, bacteria, some viruses and nematodes induce plants to form several layers of cork cells beyond the point of infection. These cork cells inhibit the further invasion by the pathogen beyond the initial lesion and also blocks the spread of toxic substances secreted by the pathogen. It also stops the flow of nutrients and water from the healthy to the infected area and deprive the pathogen nourishment. Examples of cork layer formation as a result of infection are: soft not of potato caused by *Rhizopus sp.,* potato tuber disease caused by *Rhizoctonia sp.,* Scab of potato caused by *Streptomyces scabies* and necrotic lesions on tobacco caused by tobacco mosaic virus.

Abscission layer: Abscission layers are usually formed to separate the ripe fruits and old leaves from the plant. But in some stone fruit trees, these layers develop in their young leaves in response to infection by several fungi, bacteria or viruses. An abscission layer consists of a gap formed between infected and healthy cells of leaf surrounding the locus of infection. Due to the disintegration of middle lamella of parenchymatous tissue. Gradually, infected area shrivels, dies, and sloughs off,

carrying with it the pathogen. Abscission layer formation protects the healthy leaf tissue from the attack of the pathogen. Ex: *Xanthomonas pruni* on pomegranate and *Closterosporium carpophylum* on peach leaves.

Tyloses: Tyloses are the overgrowths of the protoplast of adjacent living parenchymatous cells, which protrude into xylem vessels through pits. Tyloses have cellulosic walls. It formed quickly ahead of the pathogen and may clog the xylem vessels completely blocking the further advance of the pathogen in resistant varieties. Ex: Tyloses form in xylem vessels of most plants under invasion by the vascular wilt pathogens caused by *Fusarium oxysporum*.

Gum deposition: Various types of gums are produced by many plants around lesions after infection by pathogen or injury. Gums secretion is most common in stone fruit trees but occurs in most plants. Generally, the gum is exudate by plant under stressed condition (Tom Schultz, 2007).

Cellular defense mechanism

Hyphal sheathing: The hyphae of fungi often get enveloped in a sheath as a result of the inward stretching of the cell wall. Due to the sheathing primary delays the penetration and imparts partial check to the spread of the pathogen e.g., Late blight of potato caused by *Phytophthora infestans* and in flax infected with *Fusarium oxysporum* f.sp. *lini*.

Biochemical defense mechanism

Exudates on the surfaces of plants or compounds in plant cells may stimulate or inhibit the development of pathogens. Sometimes, plants resist infection because they do not provide the pathogen with its required nutrients. Resting spores of pathogens such as *Spongospora subterranean* (powdery scab of potato), *Urocystis agropyri* (flag or leaf smut of wheat) and *Plasmodiophora brassicae* (club root of crucifers) and eggs of the potato cyst nematode, *Globodera rostochiensis*, require specific substances to stimulate germination or hatching. These are provided in secretions from certain plants, including potential hosts. Plants that fail to secrete these stimulators are resistant by default (David G. and John 1997). Biochemical defense mechanism is mainly two type: 1. Pre-existing biochemical defense mechanism ((Phytoanticipns) and 2. Post-infectional or induced biochemical defense mechanism (Hypersensitivity response (HR) and Production of Antimicrobial substances like Phytoalexins, PR proteins and Plantibodies).

Pre-existing biochemical defense mechanism

Although structural characteristics may provide a plant with various degree of defense

against attacking pathogens. It is clear that the resistance of a plant against pathogen attack depends not so much on its structural barriers as on the substances produced in its cell before or after infection. Before infection or penetration of pathogens, host released some chemicals to defend themselves it is known as Phytoanticipns. It may be excreted into the external environment (e.g. rhizosphere or phylloplane), accumulate in dead cells or they may be sequestered in vacuoles in an inactive form.

Inhibitors present in plant cell: The plant cell contains certain pre-existing inhibitory substances which mainly play a defensive role against the particular pathogen. The antimicrobial substances which are pre-exist in plant cells includes cynogenic glycosides, sulphur containing compound, phenols and saponins (tomatin, solanin, etc.) e.g., Mustard contains isothiocynic acid, onions and garlic contains allyl sulphoxides. In *Cicer arietinum* (chickpea), the ascochyta blight resistant varieties have more glandular hairs which have maleic acid which inhibit spore germination. The dead cells of brown onion skins contain the quinones catechol and protocatechuic acid, which inhibit germination of spores of the smudge pathogen, *Colletotrichum circinans* and the neck rot pathogen, *Botrytis cinerea*. white onions do not produce these compounds and are susceptible to smudge. *Aspergillus niger* is insensitive to these inhibitors and attacks both white and brown onions. The resistance of immature apples and pears to scab, caused by *Venturia inaequalis* and *V. pirina* respectively, correlates with the presence of the phenolic compounds chlorogenic acid, phloridzin, arbutin and iso-chlorogenic acid in the outer layers of the fruit. These compounds also contribute to the bitter taste of unripe apples and pears and, as the fruit ripens and sweetens, it also becomes more susceptible to scab.

Inhibitors released by the plants: The inhibitory substances directly affect micro-organisms or encourage certain groups to dominate the environment which may act as antagonists to pathogen. Example- Tomato leaves secrete exudates which are inhibitory to *Botrytis cinerea*. Resistant varieties of linseed secrete HCN in roots which are inhibitory to linseed wilt pathogen, *Fusarium oxysporum* f.sp. *lini*. Root exudates of marigold contain α-terthinyl which is inhibitory to nematodes.

Saponins: it possesses plant glycosides with surfactant (wetting agent) properties. Saponins bind sterols in pathogen cell membranes, destroying membrane integrity and function. In this way saponins are toxic to organisms containing sterols in their membranes (e.g. plants and fungi, but not Oomycota). Inactive saponin precursor molecules appear to be stored in vacuoles of intact plant cells, but hydrolase enzymes released following wounding or infection convert these precursors to active, antimicrobial forms. Several lines of evidence suggest that saponins are involved in disease resistance and host range determination. It appears that the ability of some pathogens to detoxify specific saponins matches their host range.

For example, a strain of the take-all pathogen that attacks oats as well as wheat and barley (G*aeumannomyces graminis* var. *auenae*), releases the enzyme avenacinase. Avenacinase detoxifies the triterpenoid saponin, avenacin, found in epidermal cells of the roots of oat plants. Mutants in which the gene for avenacinase production has been deleted are sensitive to avenacin in vitro and are not pathogenic on oats, but remain pathogenic to wheat and barley. *Gaeumannomyces graminis* var. *tritici* lacks avenacinase and attacks wheat and barley, but not oat species containing avenacin. An oat species that does not produce avenacin, *Auena Longiglumis*, is susceptible to *Gaeumannomyces graminis* var. *tritici*. Another saponin, tomatine, contributes to the resistance of tomato leaves to *Botrytis cinerea*.

Lectins: Some plant peptides also inhibit the development of fungi, bacteria, viruses and insects. They act as proteinase and polygalacturonase-inhibitors, as ribosome inhibitors or lectins. These inhibitors interfere with pathogen nutrition and retard their development, thus contributing to disease resistance. Because of their similarity to peptides called defensins found in insects and mammals, they have been termed plant defensins. Secreted defensins provide an important defense against damping-off pathogens. While only 0.5% of the total protein found in ungerminated radish seeds is defensin, it makes up 30% of the proteins released from germinating seeds. It provides an antimicrobial micro-environment around the emerging radicle. Defensins may constitute up to 10% of the total proteins in cereal, legume and solanaceous seeds. Similar studies have shown defensins are also present in the outer cell layers of other plant organs such as flowers, leaves and tubers. While many defensins accumulate during normal plant development, others are induced, or their accumulation is enhanced, after wounding. Defensins, because of their anti-feeding activity against insects, provide a defense against insect-transmitted viruses.

Induced biochemical defense mechanism

Phytoalexins: One of the best and extensively-studied defense responses of plants to pathogen infection is the induced accumulation of secondary metabolites such as phytoalexins (Hammerschmidt, 1999). (Phyton = plant; alexin = to ward off). Muller and Borger first used the term phytoalexins for fungistatic compounds produced by plants in response to injury (mechanical or chemical) or infection. Phytoalexins are not produced during compatible reaction. Phytoalexins are a diverse group of low molecular weight anti-microbial compounds that are synthesized and accumulated in appreciable amounts in plants after stimulation by the various types of pathogens, by chemical or mechanical injury and are toxic to pathogens (Smith, 1996). In other words, phytoalexins are the antibiotics produced by plants that are under attack of pathogen. An effective antifungal activity towards plant pathogens is a prime and

necessary characteristic of phytoalexins. They may puncture the cell wall, delay maturation, disrupt metabolism or prevent reproduction of the pathogen. Phytoalexins are generally the products of plant metabolism which are absent form healthy plant tissue or present only in negligible traces, but accumulate in significant amounts in response to microbial attack. The biosynthesis of phytoalexins is generally expected to proceed de novo, which is from universal elementary building block such as acetate or shikimate. At least 100 plant species representing 21 families have been shown to accumulate phytoalexins in response to microbial infection and these comprise a defense mechanism analogous in some way to the immune response in animals (Yoshikawa, 1983; Keen, 1981).

Characteristics of phytoalexins: Fungitoxic and bacteriostatic at low concentrations. Produced in host plants in response to stimulus (elicitors) and metabolic products. Absent in healthy plants. Remain close to the site of infection. Produced in quantities proportionate to the size of inoculum. Produced within 12-14 hours reaching peak around 24 hours after inoculation. Host specific rather than pathogen specific.

Phytoalexin	**Host**	**Pathogen**
Pisatin	Pea	*Monilinia fructicola*
Phaseolin	French bean	Sclerotenia fructigena
Rishitin	Potato	*Phytopthora infestans*
Cicerin	Bengal gram	*Ascochyta blight*
Capsidol	Pepper	*Colletotrichum capsici*
Ipomeamarone	Sweet potato	*Ceratocystis fimbriata*
Gossipol	Cotton	*Verticillum alboratum*
Medicarpin	Alfa alfa	*Helminthosporium*
Isocoumarin	Carrot	*Cerotystis, Fusarium*
Orchinol, Hircinol	Orchid	*Rhizoctonia*

Phytoalexin detoxification

Both pathogen and plants can degrade, metabolize and sequester phytoalexin (Barz *et al.,* 1990; Moesta and Grisebach, 1982). Phytoalexin accumulation, whether biotically or abiotically induced, is not signally dependent on the rate of synthesis conjugation compartmentalization, release from conjugates, sequestering in wall or vacuoles and degradation, but all the factors in totality influence phytoalexin level It appears that phytoalexin detoxification is related to virulence in some fungi under some conditions. Transfer of pisatin demethylase to a non-pathogen of peas and the subsequent limited virulence of the non-pathogen on peas explains the role of

phytoalexins in disease resistance (Matern *et al.,* 1978). The presence of genes for detoxification of phytoalexin produced by two hosts of *Nectria hematococca*, pea and chickpea, on dispensable chromosome segments of the fungus and the retention of virulence by the fungus in the absence of the genes indicates that phytoalexins are not the only determinants of resistance in pea and chickpea.

The prior infection of potato tuber tissue with incompatible race of *Phytophthora infestans* induced resistance to a subsequent challenge by inoculation with a compatible race of *Phytophthora infestans* or tuber infecting *Fusarium* (Muller, 1958). It was hypothesized that the tuber tissue in to the incompatible interaction produced non-specific substances (phytoalexins) that inhibited further growth of the pathogen and also protected the tissue against later infection by other compatible pathogens.

Hypersensitive response (HR): In I902 Harry Marshall Ward, Professor of Botany at Cambridge University in England, observed an association between necrotic mesophyll cells in *Bromus* sp. and attempted infection of resistant cultivars by the leaf rust fungus, *Puccinia recondita*. Later E. C. Stakman at the University of Minnesota reported similar observations in resistant wheat cultivars infected with the stem rust pathogen, *P. graminis*, and in 1915 he introduced the term hypersensitivity to describe this necrotic host reaction. Stakman contended that the more resistant the cultivar, the more rapid was the collapse of host cells and the sooner the fungus was inactivated. The term hypersensitivity indicates that the host cells are 'over- (hyper-) sensitive' to the presence of the pathogen. Host cells suicide in the presence of the pathogen, preventing further spread of the infection. The most common expression of host resistance, and a frequent expression of non-host resistance, is the hypersensitive response (HR), a rapid death of cells at the infection site that is associated with pathogen limitation as well as with defense gene activation. Some of the avr genes that control the HR response to bacterial pathogens in resistant hosts also seem to act as pathogenicity (pth) genes in susceptible plants. HR occurs only in incompatible host pathogen combinations. HR is initiated by the recognition of specific pathogen produced signal molecules known as elicitors.

Once the hypersensitive response has been triggered, plant tissues may become highly resistant to a broad range of pathogens for an extended period of time. This phenomenon is called systemic acquired resistance (SAR) and represents a heightened state of readiness in which plant resources are mobilized in case of further attack. Researchers have learned to artificially trigger SAR by spraying plants with chemicals called plant activators. These substances are gaining favour in the agricultural community because they are much less toxic to humans and wildlife than fungicides or antibiotics, and their protective effects can last much longer (Brian and Gwyn, 2008). The success of hypersensitive cell death as a resistance mechanism

in individual host-parasite interactions depends on the nutritional requirements of the pathogen and on the timing, location and magnitude of the host response in relation to pathogen development. In some interactions the rapid suicide of challenged host cells undoubtedly restricts pathogen development, contributing to the overall defense response.

PR proteins (Pathogenesis Related Proteins): The survival of higher plants is dependent upon their ability to adapt to stress. Accordingly, plants have evolved to respond to stress by altering their normal patterns of gene expression (Sachs and Ho, 1986) and physiology. Plant homeostasis is affected by environmental factors such as temperature, water, salt, mechanical damage (wounding), chemicals, UV light, and the interactions with pathogenic organisms. After pathogenic attack, some plants activate the expression of "defense-related" genes (Bowles, 1990). These genes are assumed to function in the inhibition of pathogen multiplication and spread. Some defense-related genes encode enzyme involved in phenylpropanoiod metabolism (Hahlbrock and Scheel, 1989), hydrolytic enzymes (chitinases and B-1, 3-glucanases) (Boller, 1987), hydroxyproline-rich glycoproteins (cell wall proteins) (Cooper *et al.,* 1987), and proteinase inhibitors (Ryan, 1988).

Over twenty years ago it was observed that tobacco cultivars resistant to infection with tobacco mosaic virus (TMV) accumulated a new set of proteins after infection (Van Loon and Van Kammen, 1970). Subsequently, it was established that the accumulation of these proteins can also be induced by other pathogens and by the exposure to certain chemical agents. The proteins were referred to as "b" proteins or "new components". It was in 1980 when the term "pathogens-related" (PR) protein was proposed by (Antoniw *et al.,* 1980) Initial studies of the PRS were performed sing native, alkaline polyacrylamide gel electrophoresis (PAGE) system which limited the analyses to the acidic isoforms of these proteins. After the localization of PRS in the apoplastic spaces of the leaf (Parent and Asselin, 1984), they commonly become defined as host encoded, acidic, and extra cellular proteins whose synthesis were coordinately induced in pathological or related situations (chemical stress). The nomenclature used to identify them was based upon their relative electrophoretic mobility in PAGE. PRs have been identified in many dicots and monocots and appear to be ubiquitous in higher plants.

Recognized and proposed families of pathogenesis-related proteins (Van Loon and Van Strien, 1999)		
Family	**Type member**	**Properties**
PR-1	Tobacco PR-1a	Antifungal. 14-27kD
PR-2	Tobacco PR-2	Class I, II and III endo-beta-1,3 glucanases, 25-35kD
PR-3	Tobacco P, O	Class I, II, IV, V, VI and VII endochitinases, about 30kD
PR-4	Tobacco R	Antifungal, win-like proteins, endochitinase activity similar to prohevein C-terminal domain, 13-19kD
PR-5	Tobacco S	Antifungal, thaumatin-like proteins, osmotins zeamatins, permeatins, similar to alpha-amaylase/trypsin inhibitors.
PR-6	Tomato inhibitor I	Protease inhibitors, 6-13kD
PR-7	Tomato P_{69}	Endoproteases
PR-8	Cucumber chitinase	Class III chitinases, chitinase/lysozyme
PR-9	Lignin-forming peroxidase	Peroxidases, peroxidase- like proteins
PR-10	Parsley PR-1	Ribonucleases, bet v 1-related proteins
PR-11	Tobacco class V chitinase	Endochitinase activity
PR-12	Radish Ps- AFP3	Plant defensins
PR-13	Arabidopsis THI2, 1	Thionins
PR-14	Barley LTP4	Non-specific lipid transfer proteins (ns-LTPs)
PR-15	Barley OxOa (germin)	Oxalate oxidase
PR-16	Barley OxOLP	Oxalate-oxidase like proteins
PR-17	Tobacco PRp27	Unknown

Characteristics of PRs protein: PRs are distinguished by specific biochemical and structural characteristic. They are low-molecular proteins (6-43 kDa), extractable and stable at low pH (thermostable, and highly resistant to proteases. PRs have dual cellular localization - vacuolar and apoplastic, the apoplast being the main site of their accumulation (Van Loon, 1999). Apart from being present in the primary and secondary cell walls of infected plants, PRS are also found in cell wall appositions (papillae) deposited at the inner side of cell wall in response to fungal attack (Jeun, 2000). Sometimes they are also detected in the cell walls of invading fungal pathogens and in the space formed between cell walls and invaginated plasma membrane of fungi. Acidic and basic PRs have been identified, each of these counterparts with both apoplastic and vacuolar localization (Buchel and Linthorst, 1999). Earlier studies have shown that acidic tobacco PR-1 are localized in the apoplast, whereas, basic tobacco PR-1 accumulate in the vacuole (Bol *et al.,* 1990).

PRs are established in all plant organs like leaves, stem, root and flowers. In the leaves, where they can amount to 5-10% of total leaf protein. In the leaves PRS are present in mesophyll and epidermal tissues. They are also localized in the abscission

zone of leaves and inflorescence, abscission zone at the stem-petiole junction, and vascular tissue of stems and petioles (Eyal *et al.,* 1993; Del Campillo and Lewis, 1992). In inflorescences PRS are detected in sepals, pedicels, anthers, pistils, stigmata and ovaries (Van Loon, 1999; Buchel and Linthorst, 1999). In seeds of maize, sorghum, oat, barley, and wheat a group of PRS is established, commonly named permatins, characterized as PR-5 thaumatin-like proteins (Vigers *et al.,* 1991). Linusitin from flax seeds is referred to the same group (Anlovar *et al.,* 1998).

Plantibodies: Transgenic plants have been produced which are genetically engineered to incorporate into their genome, and to express foreign genes. It shown in transgenic plant. Ex:- Artichoke mottled crinkle virus.

ROS Burst: ROS are a group of free radicals, reactive molecules, and ions that are derived from O2. It has been estimated that about 1% of O2 consumed by plants is diverted to produce ROS. ROS are produced in various sub-cellular compartments such as plasma membrane, cell wall, mitochondria, chloroplasts and peroxisomes. where the large number of ROS are produced during the pathogen attack. ROS are well recognized for playing a dual role as both deleterious and beneficial species depending on their concentration in plants. At high concentration ROS cause damage to biomolecules, whereas at low/moderate concentration it acts as second messenger in intracellular signaling cascades that mediate several responses in plant cells, including stomatal closure, programmed cell death and acquisition of tolerance to both biotic and abiotic stresses. ROS include singlet oxygen (O2), superoxide anion (O2) hydrogen peroxide (H2O2) and hydroxyl radicals (OH). Hydrogen peroxide is most stable.

Conclusion

The first line of defense against the infection is the surface of the host. Since one major goal is to improve disease resistance in economically useful plants, insertion of genes encoding PR proteins, phytoalexin and other antimicrobial substances enhancing their level of expression is the method of choice. Recent advanced in molecular and analytical sciences have provided a much better view of the defense mechanism of plant against the pathogen attack. Further, studies on these strategies will continue to provide new insights into new approaches for disease management.

References

Anlover, S., Serra, M. D., Dermastia, M. and Menestrina, G. (1998). Membrane permeabilizing activity of pathogenesis-related protein lunasitin from flax seed. *Mol. Plant-Microbe Interact,* **11**: 610-617.

Antoniw, J. F., Ritter, C. E., Pierpoint, W. S. and Van Loon, L. C. (1980). Comparison

of three pathogenesis related proteins from plants of two cultivars of tobacco infected with TMV. *J. Gen. Virol.,* **47**: 79-87.

Barz, W., Bless, W., Berger, F., Gunia W. and Mackenbrock, U. (1990). Phytoalexins as the part of induced defense reactions in plants their elicitation function and metabolism. In Bioactive compounds from plants, PP. 140-56. *Ciba Found Symp.,* 154. New York Wiley.

Bol, J. F., Linthorst, H. J. M. and Cornelissen, B. J. C. (1990). Plant pathogenesis-related proteins induced by virus infection. *Annu. Rev. Phytopathol.*, **28**: 113-138.

Boller, T. (1987). Hydrolytic enzymes in plant disease resistance. In: Kosuge T, Nester E. W. (eds) Plant microbe interactions, molecular and general aspects, Vol 2 Macmillan, New York, pp: 385-413.

Bowles, D. J. (1990). Defense related proteins in higher plants. *Annu. Rev. Biochem.,* **59**:873-907.

Brian, C. F. and Gwyn, A. B. (2008). An overview of plant defense against pathogens and herbivores. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2008-0226-01

Buchel, A. S. and Linthorst, H. J. M. (1999). PR-1: A group of plant proteins induced upon pathogen infection. In: pathogenesis-related proteins in plants (eds) S. K. Datta and S. Muthukrishnan, CRC Press LLC, Boca Raton, pp: 21-47.

Cooper, J. B., Chen, J. A., Van Holst, G. J. and Varner, J. E. (1987). Hydroxyproline rich glycoproteins of plant cell walls. *Trends Biochem., Sci.,* **12**: 24-27.

David Guest and John Brown (1997). Plant pathogens and plant diseases. Rockvale publications national library of Australia ISBN 1-86389-439. Page 263-260. Edited by Brown, J. F. and Ogle, H. J.

Del Campillo, E. and Lewis, L. N. (1992). Identification and kinetics of accumulation of proteins induced by ethylene in bean abscission zone. *Plant Physiol.,* **98**: 955-961.

Eyal, Y., Meller, Y., Lev Yadun, S. and Fluhr, R. (1993). A basic-type PR-1 promoter directs ethylene responsiveness, vascular and abscission zones specific expression. *Plant J.,* **4**: 225-234.

Hahlbrock, K. and Scheel, D. (1989). Physiological and molecular biology of phenypropanoid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Bio.,* **40**:347-369.

Hammerschmidt, R. (1999). Phytoalexins: What have we learned after 60 years. *Annual Review of Phytopathology*, **37**: 285-305.

Jeun, Y. Ch. (2000). Immunolocalization of PR-protein P14 in leaves of tomato plant exhibiting systemic acquired resistance against *Phytophthora infestans* induced by pretreatment with 3-aminobutyric acid and preinoculation with TNV. *J. Plant Dis. Prot.,* **107**: 352-367.

Keen, N. T. (1981). Evaluation of the role of phytoalexins. In RC Staples, ed, Plant.

Matern, U., Strobel, G. and Shepard, J. (1978). Reactions of phytoalexins in a potato population derived from mesophyle protoplasts. *Proc. Natl. Acad. Sci.,***75**: 4935-4939.

Moesta, P. and Grisebach, H. (1982). 1-2 Aminqoxy-3-phenyipropionic acid inhibits phytoalexins accumulation in soybean with concomitant loss of resistance against *Phytophthora megasperma* f. sp. *Glycinea*. *Physiol. Plant Pathol.,* **21**: 65-70.

Muller, K. O. (1958). Studies on phytoalexins: 1. The formation and immunological significance of phytoalexins production by *Phaseolus valgaris* in response to infection with *Sclerotinia fruticola* and *Phytophthora infestans*. *Acest. J. Biol. Sci.,* **11**: 275-300.

Parent, J. G. and Asselin, A. (1984). Detection of pathogenesis related (PR or b) and of other proteins in the intercellular fluid of hypersensitive plants infected with tobacco mosaic virus. *Can. J. Bot.,* **62**:564-569.

Rayan, C. A. (1988). Proteinase inhibitor gene families: tissue specificity and regulation. In Verma, D. P. S. and Goldeberg, R. B. (eds). Temporal and spatial regulation of plant genes. Springer, Wein New York, pp: 223-233.

Sach, M. and Ho, T. H. D. (1986). Alternation of gene expression during environmental stress in plants. *Annu. Rev. Plant Physiol.,* **37**: 363-376.

Smith, C. J. (1996). Accumulation of phytoalexins: Defense mechanism and stimulus response system. *New Phytologist.,* **132**: 1-45.

Tom Schultz, (2007). Jim cooper, Master Gardener WSU County Extension, SJC © June 5, 2007

Van Loon, L. C. (1999). Occurrence and properties of plant pathogenesis-related proteins. In: pathogenesis-related protein in plants (eds) S. K. Datta and S. Muthukrishnan, CRC Press LLC, Boca Raton, pp: 1-19.

Van Loon, L. C. and Van Kammen, A. (1970). Polyacrylamide disc electrophoresis of the soluble leaf proteins from Nicotiana tabacum var. "Samsun and Samsun NN" II. Changes in protein constitution after infection with tobacco mosaic virus. *Virology,* **40**: 199-211.

Van Loon, L. C. and Van Strien, E. A. (1999). The families of pathogenesis-related

proteins, their activities and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol.,* **55**: 85-97.

Vigers, A. J., Roberts, W. K. and Sellitrennikoff, C. P. (1991). A new family of plant antifungal proteins. *Mol. Plant-Microbe Interact,* **4**: 315-323.

Yoshikawa, M. (1983). Isolation and biological activity of glycinol a pterocarpan phytoalexin synthesized by soybeans. *Plant Physiol.,* **68**: 358-368.

8 Advancements in Detecting Plant Diseases

Parmar Krishna Atulbhai

Ph.D. Scholar, N. M. College of Agriculture,
Navsari Agricultural University, Navsari - 396450, Gujarat

Abstract

Plant diseases have a significant impact on agricultural yield, leading to substantial losses. To ensure the food security of rising global population, there is a pressing need to boost production and productivity. Consequently, early detection becomes crucial in formulating effective disease management strategies. Advancements in techniques have led to a revolutionary transformation in the field of detection and diagnosis. Traditional visual inspection has given way to the adoption of advanced techniques such as advanced imaging, spectrometry, biosensors, immunological and molecular techniques. These innovative approaches offer more efficient, objective, and accurate means of identifying and managing plant diseases, ultimately contributing to sustainable agriculture, increased crop yields, and global food security.

Keywords: Disease, Detection, Biosensor, Spectroscopy, PCR, ELISA, Molecular, Serology

Introduction

Plant health is susceptible to numerous living factors, including pathogens, insect pests, weeds, human activities, as well as non-living factors like temperature, humidity, pH, and pollution. Pathogens, in particular, have a significant influence on the degradation of both the quality and quantity of plants. With the growing population, augmenting agricultural production and productivity has become crucial, and plant diseases represent a significant factor contributing to yield losses in agriculture.

To develop an effective disease management strategy, it is essential to prioritize understanding the factors responsible for causing the disease.

Plant disease detection and diagnosis involve the identification of pathogens, pests, or environmental factors that cause abnormalities in plant growth, yield, or overall health. These abnormalities may manifest as visible symptoms such as leaf discoloration, wilting, lesions, or deformities. However, symptoms alone may not provide conclusive evidence of the underlying cause, as different pathogens or stress factors can result in similar symptoms.

Traditional methods of disease detection and diagnosis have often relied on visual inspection by experienced agronomists or laboratory analysis, which can be time-consuming and subjective. To overcome these challenges, researchers and technologists have turned to innovative methods such as advanced imaging, molecular techniques, and artificial intelligence. These innovative approaches offer heightened efficiency, objectivity, and precision when it comes to the identification and management of plant diseases. In this chapter, we will explore various techniques for detecting and diagnosing different types of plant diseases.

The disease detection techniques are categorized into Indirect and Direct methods:

Indirect Methods

- Do not depend on direct identification of the presence of pathogen
- Detect the physiological and metabolic changes in plant due to infection
- Non-destructive and non-invasive method

e.g., Stress based detection techniques i.e., Imaging techniques and Bio-senser based detection techniques (Ray *et al.*, 2017)

Imaging Techniques for Plant Disease Detection

Plant disease detection using image processing techniques has gained significant attention in recent years due to its potential for early and accurate detection, which can help in timely disease management and crop protection.

These techniques can capture detailed information about plant tissues at different wavelengths or temperatures, enabling the identification of disease-specific patterns, detection of subtle changes in plant physiology and metabolism associated with diseases. Imaging techniques provide spatial information, enabling the identification of localized disease patterns across plant surfaces. The process of detecting plant diseases begins with acquiring images, followed by preprocessing

and segmentation. This is then complemented by various techniques for feature extraction and classification (Singh *et al*. 2020).

In order to identify various plant diseases, imaging systems utilize sensors to gather data for studying leaves from different perspectives. A range of valuable imaging techniques are employed for this purpose, including thermal imaging, multispectral imaging, fluorescence imaging, hyperspectral imaging, visible imaging, and magnetic resonance imaging (MRI).

1. Thermography:

It is also called as thermal imaging. The principle behind the technique involves use of infrared radiation to capture and analyze temperature distribution on the surface of the plant. As in case of plant disease infection, diseased tissues exhibit different patterns of temperature as compared to healthy tissues.

The advantages of this technique are non-invasiveness and ability of detection prior to visible symptoms development. As environmental conditions affect physiology of plants calibration is required to get accurate result (Mahlein, 2016).

2. Tomography:

It is an imaging technique which captures multiple imaging measurements from different angles around the plant and combines them to construct 3D representation of the plant's internal features through which it provides visualization and analysis of internal structures of plants.

The technique enables non-destructive examination, early detection, monitoring and characterization of plant. It may vary with target plant species and target plant disease.

3. Multispectral imaging:

It captures multiple images of plant at different wavelengths across the electromagnetic spectrum. The wavelengths are selected on the basis of the sensitivity of the plant and disease to the different parts of electromagnetic spectrum. By analysis and comparison of the characteristic spectral information associated with both healthy and diseased plant from the different bands, abnormalities, physiological changes or presence of the disease detected. Similarly, this technique also provides non-destructive, non-invasive and early disease detection result (Mahlein *et al,* 2012).

4. Hyperspectral imaging:

As healthy and diseased plants exhibit different spectral signatures, these spectral signatures correspond to the unique way in which different plant tissues and pigments interact with light. By analyzing the spectral information of plants, hyperspectral

imaging can detect subtle changes in their reflectance patterns, which can indicate the presence of diseases or stress conditions. This technique also gives non-destructive and non-invasive disease detection.

5. Fluorescent imaging:

It is widely used technique and proves highly beneficial for crop monitoring as it enables the early detection and mitigation of stress, leading to significant reductions in yield losses. It measures and analyze fluorescence emitted by plants when exposed to specific wavelengths of light. The resulting fluorescence induction curve provides valuable information about the plant's photosynthetic performance and physiological status. Changes in the shape, amplitude, and kinetics of the fluorescence induction curve can indicate the presence of stress or disease in plants.

The technique offers several advantages in plant disease detection, including high sensitivity, specificity, and the ability to visualize and quantify disease-related processes at the cellular and molecular levels.

Spectroscopic Techniques for Plant Disease Detection

Spectroscopy involves the study of the interaction between electromagnetic radiation (e.g., light) and matter (such as plant tissues). It measures how different wavelengths of light are absorbed, emitted, or scattered by the sample. Spectroscopic techniques provide valuable data about the chemical composition, molecular structures, and other characteristics of the substances being analyzed.

One prominent spectroscopic technique widely used in plant disease detection is Hyperspectral Imaging. This approach captures a spectrum for each pixel in an image, providing detailed information about the plant's biochemical and physiological status.

Another valuable spectroscopic method is Fourier Transform Infrared (FTIR) Spectroscopy. It analyzes the infrared region of the electromagnetic spectrum to identify molecular vibrations in plant tissues. FTIR has been used effectively to detect diseases like tomato yellow leaf curl virus (TYLCV) and wheat leaf rust.

Bio-Sensers for Plant Disease Detection

A biosensor is a device that combines a biological sensing element (such as enzymes, antibodies, or nucleic acids) with a transducer to detect and convert a biological response into a measurable signal. The generated signal is then transduced into an electrical, optical or chemical output, which can be measured and quantified. The sensing element of a biosensor interacts with a specific analyte, such as a protein, antigen, antibody, enzyme, or nucleic acid, resulting in a biochemical reaction.

Biosensors have proven to be valuable tools in plant disease detection and

monitoring. They offer several advantages, including rapid and sensitive detection, real-time monitoring, and the potential for on-site or in-field applications.

1. Optical biosensor:

Optical biosensors have been employed in plant disease detection due to their sensitivity, versatility, and ability to provide real-time monitoring. The principle behind optical biosensors for plant disease detection involves the interaction between a biological recognition element (e.g., antibodies, aptamers, or enzymes) and the target biomolecule (e.g., pathogen, toxin, or specific biomarker) of interest. This interaction results in a change in the optical properties of the sensing element, which is then translated into a measurable signal.

There are different optical transduction methods used in optical biosensors, and two common ones are fluorescence and surface plasmon resonance (SPR).

a. Fluorescence-based optical biosensors

The sensing element is typically labeled with a fluorescent molecule which on binding with target molecule emits fluorescence. These changes in fluorescence are detected and quantified using a fluorescence detector.

b. Surface Plasmon Resonance (SPR)-based optical biosensors

SPR occurs when polarized light interacts with a thin metal film (usually gold) on a sensor surface. When light strikes the metal surface, it induces a collective oscillation of electrons known as a surface plasmon. The angle at which this resonance occurs is highly sensitive to changes in the refractive index near the sensor surface. The sensing element is immobilized on the metal surface, and when the target biomolecule binds to the sensing element, it causes a change in the refractive index at the sensor surface. This change in refractive index results in a shift in the angle of SPR, which is detected and measured.

2. Volatile biosensor:

The principle of volatile biosensors in plant disease detection is based on the specific and selective recognition of volatile organic compounds (VOCs) emitted by plants in response to disease or stress conditions. The sensing element of the volatile biosensor is designed to have high affinity and specificity for the target VOCs. When the target VOCs are present in the air or headspace around the plant, they bind to the biological sensing element on the biosensor's surface and on binding it results in a biochemical reaction.

The biochemical reaction leads to change in properties of biosensor that generates a measurable signal that is proportional to the concentration of the

target VOCs in the plant sample. The signal is then converted and amplified by the biosensor's transducer. By comparing the VOC profile of the plant sample with known profiles from healthy and diseased plants, we can identify the presence of specific VOCs associated with the disease, which serves as an indicator for disease detection and monitoring.

Direct Methods:

- » Involves direct detection of the presence of the pathogen
- » Destructive and invasive method
- » Allows identification of non-culturable pathogens

e.g., Molecular based detection methods and Immunological based detection methods (Ray *et al*., 2017)

Molecular Techniques for Plant Disease Detection:

Another promising avenue for plant disease detection and diagnosis is the application of molecular techniques, including DNA-based methods. Polymerase Chain Reaction (PCR) and other DNA amplification techniques which can detect the presence of specific pathogens by amplifying their genetic material in plant samples.

1. Polymerase chain reaction:

It is the most powerful primer mediated technique which allows amplification and detection of specific DNA sequences associated with plant pathogens. The principle behind the technique is the ability of polymerase enzyme to synthesize new complementary strand of targeted DNA strand. The technique has been used since many years in detection and diagnosis of plant disease due to high sensitivity and accuracy.

Various methods can be used to detect the amplified PCR products. Gel electrophoresis is a common technique, where the amplified DNA fragments are separated based on size and visualized using DNA-staining dyes. Due to this drawback, more advanced PCR techniques, such as real-time PCR (qPCR), Nested PCR, Multiplex PCR, RT-PCR are used to eliminate post PCR steps.

i. Real time PCR

It is also called as Q-PCR or Quantitative PCR which monitors the amplification of DNA in real-time using fluorescent probes. A technique in which fluoro-probes, bind to specific target regions of amplicons and produce fluorescence during PCR. Thus, it enables quantitative detection and real-time monitoring of the amplification process. As it provides accurate quantification of the PCR cycles, it saves the time for post PCR procedures.

ii. Nested PCR

Nested PCR is a method that involves amplifying a specific target DNA region in two successive rounds using two different sets of primers. In the first round, a larger region of DNA is amplified, and in the second round, a smaller, internal region within the first PCR product is further amplified. Nested PCR sensitivity is 1000 times greater than single PCR for pathogen identification (Yeo and Wong, 2002). It is Used to increase the sensitivity and/or specificity of pathogen detection.

iii. Multiplex PCR

Multiplex PCR is based on the simultaneous amplification of multiple target DNA sequences using multiple sets of primers in a single reaction. It saves time and cost of the process for separate samples.

iv. RT-PCR

The technique is based on conversion of RNA into complementary DNA (cDNA) using reverse transcriptase, followed by amplification of the cDNA using PCR, enabling the detection and quantification of RNA targets.

2. Probe base method:

i. Northern blotting

Northern blot, which is also known as the RNA blot. In plant disease detection, Northern blotting is used to study gene expression patterns and identify changes in RNA levels that may be associated with specific diseases or stress conditions. It involves the detection and analysis of RNA molecules by separating them based on size through gel electrophoresis, transferring them onto a membrane, and probing with specific labeled DNA or RNA probes. This probe is typically labeled with a radioactive or fluorescent tag, which helps to detect plant diseases. It is a powerful tool for understanding the molecular basis of plant diseases and provides valuable information about the regulation of gene expression during infection or stress.

ii. In Situ Hybridization

It involves the localization and detection of specific nucleic acid sequences within cells or tissues by hybridizing them with labeled DNA or RNA probes, allowing for spatial visualization of gene expression or genetic elements. This technique allows researchers to identify the presence and abundance of target RNA sequences, such as messenger RNA (mRNA) or viral RNA, directly in the context of the plant tissue. In situ hybridization can be particularly valuable for understanding the molecular basis of plant diseases and studying the interaction between pathogens and host plants.

3. Microarray:

The principle of microarray technology involves the simultaneous detection and quantification of thousands of specific RNA molecules (mRNA) or DNA sequences in a single experiment. Microarrays consist of thousands of small DNA or RNA probes that are complementary to specific target genes or RNA sequences of interest. The cDNA samples of the target DNA are labeled with fluorescent dyes or other detectable tags, and each labeled sample is hybridized to the microarray slide containing the specific probes. After hybridization, the microarray slide is scanned to detect the fluorescence signals generated by the bound cDNA. The intensity of the fluorescence at each spot on the microarray corresponds to the expression level of the corresponding gene in the sample.

4. LAMP: Loop Mediated Isothermal Amplification

LAMP is an isothermal nucleic acid amplification technique. The technique of isothermal amplification involves the amplification of DNA or RNA sequences under constant temperature conditions, eliminating the need for thermal cycling, and enabling rapid and efficient nucleic acid amplification. The principle of LAMP relies on the use of multiple primers and a DNA polymerase with strand displacement activity to amplify the target DNA under isothermal conditions. The amplification products are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops. The presence of amplified DNA can be detected using various methods, such as visual observation of turbidity or by adding fluorescent dyes that bind to double-stranded DNA.

Immunological Techniques for Plant Disease Detection

When the body or a plant is exposed to foreign substances, such as antigens, a response is triggered, leading to the production of specific protein molecules known as antibodies. Serological detection techniques operate based on the principle of utilizing the specificity of antibodies or antigens. It employs the binding between antibodies and antigens to detect and measure the presence of specific pathogens in a sample. There are various serological techniques developed such as Immunodiffusion test, ELISA (enzyme linked immunosorbent assay), DIBA (dot immunobinding assay), TBIA (tissue blot immunoassay), Immunosorbent electron microscopy and Flow cytometry.

1. Immunodiffusion test

The immuno-diffusion test, also known as the agar gel immunodiffusion (AGID) test, is a simple and widely used serological technique for detecting the presence of specific antibodies or antigens in a sample. The immuno-diffusion test is based

on the principle of antigen-antibody interactions and their ability to form visible precipitin lines when allowed to diffuse through an agar gel medium.

If the antigen and antibody are specific to each other (i.e., they react due to the presence of the target pathogen or plant-specific antigens), they will form immune complexes by combining in the gel medium. As the immune complexes diffuse outward from their respective wells, they meet and form visible precipitin lines where the antigen and antibody react. The appearance of precipitin lines indicates a positive reaction, suggesting the presence of the target pathogen or plant-specific antibodies in the sample.

2. ELISA (Enzyme Linked Immunosorbent Assay)

ELISA is a powerful tool for rapid and sensitive detection of plant pathogens, and it is commonly employed in both research and agricultural settings for disease diagnosis and monitoring. It is based on the principle of specific antigen-antibody interactions, allowing the detection and quantification of target pathogens or plant-specific antigens in plant samples. In ELISA, two antibodies are used: primary and secondary. Primary antibody is coated to the microtiter plate whereas secondary antibody is used as detection antibody which is labelled with enzymes. This enzyme on attachment to the antigen-antibody complex, produces signal. The intensity of the signal is measured which is directly proportional to the amount of target antigen or antibody present in the sample.

3. DIBA (Dot immunobinding assay)

Dot immunobinding assay is a simplified and rapid immunological technique used to detect the presence of specific antigens or antibodies in a sample. The principle of Dot-IBA is similar to other immunobinding assays, such as the Enzyme-Linked Immunosorbent Assay (ELISA) but it involves the application of the sample as small dots on a solid support, such as a nitrocellulose or PVDF membrane.

4. TBIA (Tissue blot immunoassay)

The Tissue Blot Immunobinding Assay (TBIA) is a serological technique used in plant disease detection to identify the presence and distribution of specific pathogens, such as viruses or bacteria, in plant tissues. TBIA allows researchers to visualize the spatial distribution of the pathogen within the plant, which can aid in disease diagnosis and monitoring. By visualizing the spatial distribution of the pathogen in the plant, TBIA can help differentiate localized infections from systemic ones and identify the extent of disease spread.

5. Immunosorbent Electron Microscopy

Immunosorbent Electron Microscopy is a combination of immunological techniques

and electron microscopy. It involves the use of antibodies or antigens conjugated with electron-dense markers (such as gold particles) to visualize specific interactions between antigens and antibodies at the ultrastructural level. Immunosorbent Electron Microscopy (ISEM) is a highly specialized technique that provides detailed information about the ultrastructural localization of specific antigens or pathogens within plant tissues. It can be valuable for studying the interactions between pathogens and host plants, understanding disease mechanisms, and characterizing disease symptoms at the cellular and subcellular levels.

6. Flow cytometry

Flow cytometry is a powerful analytical technique used in plant disease detection and research to analyze and quantify various characteristics of individual plant cells or particles in a liquid suspension. The principle of flow cytometry involves the passage of individual plant cells or particles through a flow cell in a liquid stream. As the cells pass through the flow cell, they are exposed to a laser beam, and the light scattered or emitted by the cells is measured. Additionally, fluorescent markers or dyes can be used to label specific cellular components, such as DNA, proteins, or organelles, enabling the detection of specific features or molecules within the cells. The light scattered by the cells and the emitted fluorescence are detected by photodetectors. It allows for the rapid and simultaneous measurement of multiple parameters, providing valuable information about the physiological and pathological changes occurring in plant cells during disease development.

Conclusion

In conclusion, plant disease detection techniques play a crucial role in safeguarding agricultural productivity and ensuring food security. Over the years, various advanced and innovative methods have been developed to accurately and rapidly identify pathogens, assess disease severity, and monitor plant health. These techniques include serological assays like ELISA and immunoblotting, molecular methods like PCR and LAMP, imaging-based approaches like hyperspectral imaging.

The diversity of these techniques allows researchers, plant pathologists, and farmers to choose the most appropriate approach for their specific needs, considering factors such as sensitivity, specificity, cost, and ease of use. Combining multiple approaches can enhance disease detection accuracy and provide comprehensive insights into plant-pathogen interactions.

As technology continues to advance, we can expect further improvements and innovations in plant disease detection. These include the integration of artificial intelligence, machine learning, and bioinformatics to enhance data analysis and

interpretation, the utilization of portable and field-deployable devices for on-site diagnostics, and the continuous improvement of high-throughput sequencing technologies for comprehensive pathogen profiling.

References

Das, A., and Mohanta, R. (2017). Image processing techniques for disease spot detection on plant leaves: A survey. *International Journal of Computer Science and Information Security*, 15(6):109-114.

Ghosal, S., Blystone, D., Singh, A. K., Ganapathysubramanian, B., Singh, A. and Sarkar, S. (2018). An explainable deep machine vision framework for plant stress phenotyping. *Proceedings of the National Academy of Sciences*, 115(18):4613-4618.

Haralick, R. M., Shanmugam, K. and Dinstein, I. (1973). Textural features for image classification. *IEEE Transactions on systems, man, and cybernetics*, (6):610-621.

Mahlein, A. K., Steiner, U., Hillnhütter, C., Dehne, H. W., & Oerke, E. C. (2012). Hyperspectral imaging for small-scale analysis of symptoms caused by different sugar beet diseases. *Plant Methods*, 8(1):3.

Mahlein, A. K. (2016). Plant disease detection by imaging sensors – parallels and specific demands for precision agriculture and plant phenotyping. *Plant Disease*, 100(2), 241-251.

Nanni, L., Ghidoni, S. and Brahnam, S. (2018). A survey on image fusion applications in agriculture. *Information Fusion*, 43:42-54.

Ray, M., Ray, A., Dash, S., Mishra, A., Achary, K. G., Nayak, S. and Singh, S. (2017). Fungal disease detection in plants: Traditional assays, novel diagnostic techniques and biosensors. *Biosensors and Bioelectronics,* 87:708–723.

Sladojevic, S., Arsenovic, M., Anderla, A. and Culibrk, D. (2016). Deep neural networks-based recognition of plant diseases by leaf image classification. *Computational intelligence and neuroscience*, 2016.

Singh, V., Sharma. N and Singh. S. (2021). A review of imaging techniques for plant disease detection. *Artificial Intelligence in Agriculture*, 5:301-302.

Yeo, S. F. and Wong, B. (2002). Current Status of Nonculture Methods for Diagnosis of Invasive Fungal Infections. *Clinical Microbiology Reviews*, 15(3):465– 484.

9 Technology for Mass Production of *Trichoderma* spp.

C. M. Bhaliya[1], H. A. Shekhada[2], R. L. Joshi[3] and J. J. Padsala[4]

[1]Assistant Research Scientist, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, (Junagadh) - 362 001
[2]Ph.D. Scholar, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, (Junagadh) 362 001
[3]Ph.D. Scholar, Navsari Agricultural University, (Navsari) 369450
[4]Ph.D. Scholar, Navsari Agricultural University, (Navsari) 369450

Abstract

Trichoderma spp. have emerged as potent biocontrol agents and plant growth promoters with immense potential for sustainable agriculture and disease management. The mass production of these beneficial fungi is essential to meet the increasing demand for their application in modern farming practices. The mass production of *Trichoderma* is commonly achieved through solid and liquid state fermentation methods. While solid fermentation can be expensive due to substrate requirements, liquid fermentation using cost-effective media like molasses and yeast medium is the preferred approach in commercial production. *Trichoderma* formulations are available in various forms, such as dusts, granules, pellets, and wettable powders. These formulations are directly applied to the soil in nurseries and main fields to prevent soil-borne pathogen inoculum. Seed dressing with *Trichoderma* formulations through dry seed treatment or seed biopriming is an effective strategy to protect seeds from soil-borne diseases during germination. In field conditions, adding *Trichoderma*-enriched farmyard manure (FYM) directly to the soil has proven to be an efficient method for disease management in both nurseries and fields. Granular or pellet preparations of *Trichoderma* are also viable options for application. To ensure the safety and efficacy of *Trichoderma* products, thorough testing and quality control measures are essential. Ensuring these products do not

harm humans, the environment, or other living organisms is critical. Strict quality standards must be maintained to prevent the sale of poor-quality goods to farmers. Ensuring the safety and quality of *Trichoderma* products is paramount for successful disease control and sustainable agriculture.

Keywords: *Trichoderma*, mass multiplication, formulation, seed dressing, soil borne.

Introduction

The green revolution brought about a significant increase in agricultural productivity through the extensive use of chemical fertilizers and insecticides. However, the long-term and widespread application of chemical biocides to combat various organisms, including weeds, fungi, and insects, has raised concerns regarding their potential impact on human health and the environment. As a result, there is a global push to minimize the use of chemical pesticides, paving the way for the development of sustainable crop protection techniques.

Biological control using microorganisms has emerged as a natural and environmentally acceptable alternative to chemical treatments. Among these microorganisms, *Trichoderma*, an antagonistic fungus found in soil and root ecosystems, stands out as a potent biocontrol agent. *Trichoderma* species have been known to protect crops from a wide range of soil and seed-borne diseases, making it particularly effective in various agricultural settings.

The diverse species of *Trichoderma*, such as *T. virens, T. viride, T. koningii, T. polysporum, T. hamatum, T. longibrachiatum, T. asperellum,* and *T. harzianum* are widely utilized for controlling plant diseases. *Trichoderma* employs various regulatory mechanisms to combat phytopathogens, including competition, mycoparasitism, antibiosis, synthesis of lytic enzymes, and release of secondary metabolites. Moreover, it promotes plant growth and development, enhances nutrient uptake, and boosts stress tolerance (Woo *et al.,* 2014). It has high rates of sporulation and recombination, as well as high levels of competitiveness and saprophytic survival. (Howell, 2013). Its different strains grow rapidly when inoculated in the soil, since they are naturally resistant to many toxic compounds, including herbicides, fungicides and pesticides (Seethapathy *et al.,* 2017).

Mass production of *Trichoderma* has become a significant area of research in search of alternatives to chemical pesticides and fertilizers (Parkash and Saikia, 2015). *Trichoderma*'s ability to resist toxic compounds, including herbicides and fungicides, makes it an appealing choice for commercial usage. However, high costs associated with traditional synthetic media used for production limit its widespread commercial adoption (Subash *et al.,* 2013). The research is focused on finding cost-

effective alternatives for *Trichoderma* mass production, exploring substrates like home waste, vegetable waste, and other biodegradable materials. Successful formulations must meet specific criteria, such as low application dose, long shelf life, ease of use, contaminant-free composition, and economic viability for commercial profitability (Babu and Pallavi, 2013).

The efficient formulation and delivery mechanisms for bioagents are crucial for implementing biological control through microbial antagonists. The commercially available *Trichoderma* formulations are used to manage various plant diseases, but there is a need for multiplying *Trichoderma* spp. on biodegradable substrates with extended shelf life for commercial-scale usage.

The search for sustainable alternatives to chemical pesticides has highlighted the potential of *Trichoderma* spp. as a highly effective biocontrol agent for managing plant diseases and promoting crop growth. The challenge lies in developing cost-effective mass production technologies and viable formulations for its commercial utilization. By addressing these challenges, *Trichoderma*-based biocontrol can contribute significantly to sustainable agriculture and reduce the environmental impact of traditional chemical-intensive practices.

Mode of action

The success of any bioagents is depends on their high reproductive capacity, capacity to survive in harsh conditions, efficiency in nutrient utilization, capacity to modify the rhizosphere, aggressiveness against phytopathogen, and potential in promoting plant growth and defense mechanisms. *Trichoderma* is a widespread genus with enormous population densities that can be found in any habitat. The *Trichoderma* suppress the pathogen by using the following various modes of action.

(A) Competition

It is a phenomenon where the pathogen and the newly introduced biocontrol agent (antagonist) compete for resources and space. The antagonist may inhibit the expansion of the pathogen population in the rhizosphere during this process, hence preventing the onset of disease. The nutrient-poor conditions are typically seen in soils and on the surfaces of plant growth. Evidence suggests that managing the incidence and severity of disease depends upon competition between pathogens and non-pathogens for nutrient resources. In opposition to diseases that germinate directly on plant surfaces and infect through appressoria and infection pegs, soil-borne pathogens that infect through mycelial contact are typically more vulnerable to competition from other soil- and plant-associated pathogens. The most prevalent nonpathogenic plant-associated microorganisms are typically believed to protect

the plant by aggressively colonizing the minimal substrates that are available and preventing any from being left for pathogens to develop on. These microorganisms produce additionally compounds that inhibit pathogens.

(B) Antibiosis

Indeed, many microorganisms, including Trichoderma species, are known to produce and secrete substances with antibiotic properties. These compounds play a significant role in biocontrol, as they help inhibit the growth and colonization of plant pathogens, leading to disease management. Trichoderma strains have been extensively studied for their ability to produce a variety of volatile and nonvolatile substances that exhibit antimicrobial activity. Some of the key compounds produced by *Trichoderma* include harzianic acid, alamanthicins, tricholin, peptaibol antibiotics, massoilacetone, 6-pethyl-a-pyron, viridian, glioviridin, gliosporenins, heptelidic, and other metabolites have been described as compounds produced from these metabolites.

These compounds help *Trichoderma* compete with and suppress plant pathogens, making it an effective biocontrol agent for disease management in agriculture and horticulture. *Trichoderma*'s ability to produce a wide array of antimicrobial substances is one of the reasons it has been extensively studied and utilized for sustainable plant protection strategies.

(C) Mycoparasitism

The infection of other fungi by the antagonistic fungi is known as mycoparasitism. The mechanism involves different stages of interaction. The antagonist fungi are attracted to the pathogenic fungi by their chemical stimulus, which results in a chemotropic response from the antagonist. In second stage, lectins assist in identifying between the pathogen and the antagonist. In third stage, the interactions between the antagonist and the pathogen's hyphae come next. The antagonist (*Trichoderma*) hyphae either grow beside the host hyphae or coil around it and secrete several lytic enzymes (chitinase, glucanase, and pectinase) that are involved in the mycoparasitism process.

(D) Triggering of plant defense mechanisms

1. **Induction of Systemic Acquired Resistance (SAR):** *Trichoderma* can activate the systemic acquired resistance (SAR) pathway in plants. This response is triggered when the plant perceives the presence of *Trichoderma* or other beneficial microbes. The activation of SAR leads to the accumulation of pathogenesis-related (PR) proteins, which play a crucial role in plant defense against pathogens. These PR proteins make the plant more resistant to various pathogens and help combat potential infections effectively.

2. **Induced Systemic Resistance (ISR):** *Trichoderma* can also induce induced systemic resistance (ISR) in plants. Through interactions with the plant's roots and the rhizosphere, *Trichoderma* can trigger the production of jasmonic acid (JA) and/or ethylene in the plant. These signaling molecules then activate ISR, which primes the plant's immune system for a more rapid and effective response to potential pathogen attacks. ISR does not involve the accumulation of PR proteins but still enhances the plant's defense capabilities.

(E) Promotion of Plant Growth

Trichoderma strains are characterized as symbiotic, opportunistic, avirulent organisms that can colonize plant roots by a process similar to that of mycorhizal fungi and produce substances that promote plant growth and development and defense mechanisms. *Trichoderma* strains frequently improve root growth and development, crop productivity, resistance to abiotic stresses, and nutrient uptake (Harman *et al.* 2004).

1. **Root growth promotion:** *Trichoderma* can stimulate root growth and branching, leading to a more extensive and robust root system. A well-developed root system enhances nutrient and water uptake by the plant, improving overall plant health and growth.
2. **Nutrient solubilization:** *Trichoderma* possesses the ability to solubilize certain nutrients, such as phosphorus and iron, in the soil. This process makes these nutrients more available to the plant, leading to improved nutrient uptake and utilization.
3. **Phytohormone production:** *Trichoderma* can produce and release phytohormones like auxins and gibberellins, which influence various aspects of plant growth and development, such as cell elongation and seed germination.
4. **Enhanced stress tolerance:** *Trichoderma* treated plants often exhibit increased resistance to abiotic stresses, such as drought, salinity, and extreme temperatures. The presence of *Trichoderma* induces changes in the plant's physiology and metabolism, making it more resilient to adverse environmental conditions

Why *Trichoderma* spp. act as ideal bioagents (Jeyarajan and Nakkeeran 2000)

The following characteristics makes *Trichoderma* spp. as an ideal bioagent;

i. High rhizosphere competence

ii. Highly competitive saprophytic ability

iii. Enhanced plant growth
iv. Ease for mass multiplication
v. Broad spectrum of action
vi. Excellent and reliable control
vii. Safe to environment
viii. Compatible with other bioagents
ix. Tolerate to desiccation, heat, oxidizing agents and UV radiations

Mass multiplication techniques

The biological control is an effective and eco-friendly approach for controlling many plant diseases. Further, biological control strategy is highly compatible with sustainable agriculture and is an important component of integrated pest management (IPM) programme. The success of biological control depends on mass production of the biocontrol agent in laboratory. The large scale availability of the pathogen is a primary requirement in the biocontrol programmed. For a successful integrated pest management programme, the bioagents should be amenable to easy and cheap mass multiplication. The mass production of bioagent is achieved through solid and liquid fermentation techniques.

1. Solid state fermentation technique

It is a mass multiplication process of bioagents in which insoluble materials in water is used for the microbial growth. It is an effective method for the mass production of fungal bioagents, since it provides large amount of biomass (mycelium or spore). In this method, various cheap cereal grains *such as;* sorghum, millets, ragi are utilized as substrates.

2. Liquid state fermentation technique

It is a mass multiplication process of bioagents in which soluble materials in water is used for the microbial growth. It is an effective method for the mass production of fungal as well as bacterial bioagents. In this method, Potato dextrose broth, V-8 juice, Molasses-yeast medium are generally used for the mass production of bioagents.

Mass Multiplication of Fungal Bioagent- *Trichoderma* sp.

Isolation of *Trichoderma* sp. from soil

» Trichoderma is isolated from the soil by using serial dilution technique in laboratory.

» Collect soil samples from the field, mix well and make it into fine particles.

Soil samples should be collected in root zone at 5-15 cm depth and from rhizosphere wherever possible.

» Ten gram of soil sample is taken and suspended in 100 ml of sterile distilled water and stirred well to get uniform dilution.

» Transfer one ml from this to 9 ml of sterile water in a test tube to get 1: 100 (10^{-2}) dilution. Make serial dilutions by transferring one ml of suspension to subsequent tubes to get dilution of 1:10,000 (10^{-4}).

» Transfer one ml of the desired soil suspension to sterile petriplates. Pour 15 ml of sterilized, melted and cooled *Trichoderma* selective medium in the same petriplates.

» Rotate the plate gently and allow to solidify, incubate at room temperature for 5-7 days and observe for the development of fungal colonies.

» *Trichoderma* colonies will be white initially and turn to green. Count the number of colonies developing in individual plates. Transfer the individual colonies to potato dextrose agar slants for future use.

» After getting the pure culture of *Trichoderma,* it can be mass multiply by using following methods.

(A) Solid state fermentation

Procedure

1. Cheap cereal grains *such as;* sorghum, millets, ragi are used to prepare pure powder of *Trichoderma.*
2. Wash and soak the grains in water for 12 hrs. or give 2-3 water wash to the grain after washing boil the grain up to half cooked, to avoid contamination add antibiotics in it after that filled in 250 ml flasks @ 100g/flask or in half kg polypropylene bags @ 150 g /bag.
3. Plug the flasks and bags with non-absorbent cotton plug (with the help of plastic pipe neck). Autoclave it at 121^0 C temperature and 15 lbs psi for 15 min.
4. After cooling to room temperature, inoculate the flasks or bags with 4-6 bits of actively growing culture of *Trichoderma* sp. from pure culture. Incubate the bags at 28^0C for 4-5 days. Incubation period may be varied depending upon green sporulation developed in bag/flask.
5. After few days, the colonized grains turn into green color due to spore produce by the fungi. Then colonized grains are emptied into plastic trays

and sieved through a normal coarse (50 mesh size) and fine (80 mesh size) sieves, simultaneously to obtain a very fine pure powder.

6. This pure powder is later mixed with talcum powder in a ratio of 10 g pure powder + 1 kg talc powder (commercial grade) to get the commercial formulation. 10% Carboxy Methyl Cellulose (CMC) is also added as a sticker.
7. It is packed in polythene beg, keep it in cool and dry place and utilized it within 4 months.

(b) Liquid state fermentation

Procedure

1. Molasses yeast medium good for liquid fermentation. Prepare molasses yeast medium (molasses-30 gram, yeast powder-5 gram, distilled water 1000 ml) by mixing the ingredients of respective medium in conical flasks/glass bottles and sterilized it at 121^0 C temperature and 15 lbs psi for 15 min.
2. After cooling at room temperature, inoculate this medium with mycelia and spore bits of *Trichoderma* sp. from 10 day old pure culture.
3. Incubate the flasks at room temperature either as stationary culture for 3-4 days or on a rotary shaker at 150-180 rpm for 2-3 days. Incubation period may be varied depending upon the mycelia mat and spores developed in medium. This is serves as mother culture.
4. Generally, molasses yeast medium is prepared in an automatic fermenter and sterilized. Then, the mother culture which is prepared earlier is added to the fermenter @ 1.5 liter/50 liters of medium and incubated at room temperature for 10 days.
5. After the development of mycelia mat and spores in broth. The fungal biomass and broth are directly mixed with talc powder at 1:2 ratio. The mixture is air dried and mixed with carboxy methyl cellulose (CMC) @ 10 g/ kg of the product.
6. Later, this mixed product is packed in polythene beg keep it in cool and dry place and utilized it within 4 months.

Advantage of liquid carrier media

Using liquid carrier media for mass multiplication of *Trichoderma* offers several advantages over other methods, such as solid-state fermentation. Some of the key advantages include,

1. **Homogeneous and consistent growth:** Liquid carrier media provide a well-mixed and uniform environment for *Trichoderma* growth. This ensures consistent propagation of the fungus throughout the medium, resulting in a higher yield of viable propagules.
2. **Higher spore production:** Liquid media generally promote higher spore production compared to solid media. This is beneficial when aiming for mass multiplication of *Trichoderma*, as a higher spore count means more efficient disease control and better results in plant growth promotion.
3. **Easy scalability:** Liquid fermentation is easily scalable, making it suitable for large-scale production of *Trichoderma*. It allows for the production of substantial quantities of biopesticides or biofungicides to meet agricultural demands
4. **Enhanced nutrient availability:** The nutrients in liquid carrier media are readily available to the *Trichoderma* culture, promoting rapid growth and increasing the biomass production. This leads to a higher concentration of beneficial metabolites and enzymes produced by *Trichoderma* that contribute to disease suppression and plant growth stimulation.
5. **Improved aeration and oxygen supply:** Liquid media provide better aeration and oxygen supply to the *Trichoderma* culture compared to solid media. Sufficient oxygen is crucial for aerobic fermentation and the growth of *Trichoderma*, leading to healthier and more vigorous cultures.
6. **Efficient nutrient uptake:** The nutrients in liquid carrier media are typically more accessible to *Trichoderma*, leading to more efficient nutrient uptake and utilization. This results in faster growth and higher yields of viable spores.
7. **Easy product recovery:** Harvesting *Trichoderma* from liquid media is often simpler and less labor-intensive than from solid substrates. The liquid fermentation process allows for easier separation of the fungal biomass from the liquid, simplifying the downstream processing steps.
8. **Reduced contamination risk:** Liquid media can be sterilized more effectively than solid media, reducing the risk of contamination from unwanted microorganisms. This is essential for maintaining the purity and efficacy of the *Trichoderma* biopesticides or biofungicides.

The use of affordable and readily available solid materials as substrate substitutes for synthetic solid media in *Trichoderma* mass multiplication is a practical and sustainable approach. As mentioned, traditional substrates like glucose, cellulose, soluble starch, and molasses can be scarce and expensive, making them less practical for large-scale production (Gupta *et al.* 1997). By utilizing materials such as sawdust,

rotting wheat grains, rice husk, and vegetable waste, researchers can not only reduce production costs but also make use of agricultural and food industry by-products, turning them into valuable resources (Khan *et al.* 2011). This approach aligns with the principles of circular economy and waste reduction.

However, it is important to acknowledge that solid-state fermentation using these natural substrates has its limitations, as stated in the disadvantages. These include the need for a larger amount of substrate compared to liquid fermentation, which may be an issue for some applications where cost-effectiveness and production scale are critical factors. Contamination is another concern with solid-state fermentation.

Although using natural substrates can contribute to nutrient diversity and help establish a favorable microbial community, it also creates a risk of unwanted microorganisms colonizing the medium, potentially reducing the efficacy and consistency of the final *Trichoderma* product. Extended fermentation times are often associated with solid-state fermentation due to the slower diffusion of nutrients and oxygen into the substrates. This can be a drawback when a rapid production cycle is required

On the other hand, liquid fermentation offers various advantages. As mentioned earlier, it provides a more homogeneous and nutrient-rich environment, leading to higher spore production and faster growth. It is also easier to control and sterilize liquid media, reducing contamination risks. Additionally, the ease of handling and scalability of liquid fermentation make it a favorable choice for mass multiplication.

Different formulation of *Trichoderma*

1. Talc based formulation

Trichoderma is grown in a liquid medium, incorporated into tale powder in a 1:2 ratio, and dried to an 8% moisture level while being shaded. This formulation had a three to four-month shelf life. In India, treating seeds at a rate of 4 to 5 g/kg seed has proved highly effective for controlling a number of soil-borne diseases that affect a variety of crops (Jeyarajan, 2006) (Jeyarajan *et al.* 1994).

2. Oil-based formulation

They are created by creating a stable emulsion by mixing the conidia collected from the solid state/liquid state fermentation with a combination of vegetable/mineral oils. In such formulations, microbial agents are suspended with the help of a surface-active substance in a water-immiscible solvent such as a petroleum fraction (diesel, mineral oils), and vegetable oils (groundnut, etc.). This can be mixed with water to create an emulsion that is stable. For quickly homogenous emulsion formation upon dilution in water, emulsifiable concentrates require a high concentration of an oil soluble

emulsifying ingredient. The oils utilized shouldn't be harmful to plants, humans, animals, or spores of fungus. These *Trichoderma* formulations are right now applied directly as sprays. Oil-based formulations are considered to have an extended shelf life and are appropriate for foliar sprays in dry weather. The oil coating the spores protects them from drying out and lets them to survive longer on the plant surface even in dry weather. For the sole purpose of combating *Botrytis cinerea* post-harvest apple rot, *T. harzianum* emulsion formulation has been developed. At the earlier Project Directorate of Biological Control (PDBC) in India, an invert-emulsion formulation of *T. harzianum* with an 8-month shelf life has been developed using ingredients that are accessible locally. This formulation has been evaluated and found to be effective against groundnut diseases that are spread through the soil (Batta, 2005).

3. Pesta granules based formulation

In pesta granules formulation, 100 g of wheat flour and 52 ml of fermenter biomass are mixed with gloved hands to produce a cohesive dough. Hand actions are used to frequently squeeze, fold, and knead the dough. After that, one mm thick sheets (known as pesta) are made and air dried till they break easily. Following drying, the dough sheet was broken down and fed through an 18-mesh screen, and the granules were gathered (Connick *et al.* 1991).

4. Alginate prills based formulation

Food base is suspended in another portion (50 g/250 ml), while sodium alginate is dissolved in one piece of distilled water (25g/750ml). These preparations are autoclaved, cooled, and then combined with biomass. To create spherical beads that are air-dried and kept at 5°C, the mixture is added drop by drop to the $CaCl_2$ solution (Fravel *et al.* 1999).

5. Vermiculite wheat bran-based formulation

For 10 days, *Trichoderma* is multiplied in molasses-yeast media. 33 g of wheat bran and 100 g of vermiculite are sterilized for three days at 70 °C in an oven. Then, 20 g of fermenter biomass, 0.05 N medium, and concentrated or naturally derived biomass with HCI are added, thoroughly combined, and then dried in the shade (Lewis, 1991).

6. Coffee husk-based formulation

A *Trichoderma* mixture was created using coffee husk, a byproduct of the coffee curing business. This product, which is popular in Kerala and Karnataka, was very successful in treating Phytophthora foot rot of black pepper (Sawant and Sawant 1996).

7. Press mud-based formulation

The sugar factory produces press mud, which can be utilized as a substrate for *Trichoderma* to multiply in large quantities. The process involves evenly blending a 9-day-old *T. viride* culture grown in potato dextrose broth with 120 kg of press mud. To keep it moist, water was intermittently poured in. Gunny bags were used to cover this in order to allow air and trap moisture in the shade. Nucleus culture for subsequent multiplication is ready in 25 days. The same mixture was mixed completely, added to 8 tons of press mud, and then incubated for 8 days in the shade before being used in the field. By doing this, we increased the number of inoculums in the soil by 8000 times over the doses of biopesticides that are recommended causing them to have immediate impact. Similar to this, additional substances could also be utilized in an efficient manner for the mass-level multiplication of various bioagents (Sabalpara, 2014).

Shelf life of *Trichoderma*

Trichoderma based formulations or biofungicides are widely used in agriculture and horticulture to control various plant diseases and promote plant growth. The shelf life of a *Trichoderma* formulation refers to the duration for which the product remains viable and effective when stored under appropriate conditions. The shelf life of *Trichoderma* formulations can vary depending on several factors, including the specific *Trichoderma* species used, the formulation type (powder, granules, liquid, *etc.*), the manufacturing process, and the storage conditions. Typically, shelf life is measured in terms of the number of viable colony-forming units (CFUs) present in the product.

For most *Trichoderma* formulations, the shelf life ranges from six months to two years from the date of manufacture. More than 18 months shelf life of *Trichoderma* observed in coffee husk. *Trichoderma* formulations with a talc, peat, lignite, or kaolin base have a three to four-month shelf life. (Sankar and Jeyarajan 1996). However, to ensure optimal performance, it is crucial to store the product correctly. *Trichoderma* formulations should be kept in a cool, dry place, away from direct sunlight and extreme temperatures, as high temperatures and humidity can significantly reduce the viability of the fungal spores. As the product ages, the number of viable spores in the formulation may gradually decline. The population of *T. viride* appears to be most prevalent in milky white bags with a 100-micron thickness. Consequently, using older *Trichoderma* formulations might result in reduced efficacy in disease control and plant growth promotion.

It is advisable to check the expiration date before purchasing and to use the product within the recommended period to achieve the best results. Regularly updating Trichoderma products and adhering to proper storage practices can help

maximize their benefits in sustainable agriculture and disease management. The work on prolonging the shelf life of *Trichoderma* formulations has been carried out at PDBC Bangalore using various components (chitin and glycerol) in the production medium and heat shock at the end of the log phase of fermentation. This procedure can extend the shelf life of *Trichoderma* formulations by up to a year. (Sriram *et al.* 2010 and 2011).

Characteristics of an ideal formulation of *Trichoderma*

An ideal formulation of *Trichoderma*, a beneficial fungus used in agriculture and horticulture for its biocontrol and plant growth-promoting properties, should possess the following characteristics

1. **High viability:** The formulation should contain live and viable *Trichoderma* spores that remain effective during storage and application. High spore viability ensures better performance in the field.
2. **High population density:** The formulation should have a high concentration of *Trichoderma* spores to ensure effective colonization and rapid establishment on plant roots and in the rhizosphere.
3. **Broad spectrum of activity:** The ideal formulation should be capable of combating a wide range of plant pathogens, including various types of fungi, bacteria, and nematodes. This broad-spectrum activity allows it to protect plants from different diseases
4. **Compatibility with other products:** The formulation should be compatible with commonly used agricultural inputs, such as fertilizers and pesticides. This ensures that Trichoderma can be integrated into existing farming practices without adverse effects on other beneficial organisms
5. **Long shelf life:** Stability during storage is crucial for commercial viability. The formulation should have a long shelf life while maintaining its efficacy
6. **Easy application:** The formulation should be user-friendly and easy to apply through various methods, such as seed treatment, soil application, or foliar spray
7. **Resistance to environmental stress:** *Trichoderma* should be able to tolerate a wide range of environmental conditions, including temperature fluctuations, UV radiation, and soil pH variations, to remain active and effective in different climates and soil types
8. **Safe for non-target organisms:** An ideal *Trichoderma* formulation should be harmless to non-target organisms, including beneficial insects, earthworms, and other microorganisms in the soil.

9. **Enhanced plant growth promotion:** Apart from disease suppression, the formulation should promote plant growth by producing growth-promoting substances, stimulating root development, and enhancing nutrient uptake.
10. **Ecologically friendly:** The formulation should be environmentally friendly, biodegradable, and not contribute to pollution or harmful residues.
11. **Scientifically proven efficacy:** The effectiveness of the formulation should be supported by scientific research and field trials to ensure farmers' confidence in its performance.
12. **Cost-effective:** The formulation should be economically feasible and provide a cost-effective solution for farmers to improve crop health and yield.

Overall, an ideal formulation of *Trichoderma* should combine effective disease control with plant growth promotion, safety for the environment, and ease of use to be widely adopted in sustainable agriculture practices.

Different method for *Trichoderma* application

1. **Seed treatment:** Mix 6 - 10 g of *Trichoderma* powder per kg of seed before sowing.
2. **Nursery treatment:** Apply 10 - 25 g of *Trichoderma* powder per 100 m^2 of nursery bed. Application of neem cake and FYM before treatment increases the efficacy.
3. **Cutting and seedling root dip:** Mix 10 g of *Trichoderma* powder along with 100 g of well-decomposed FYM per liter of water and dip the cuttings and seedlings for 10 minutes before planting.
4. **Soil treatment:** Apply 5 kg of *Trichoderma* powder per hectare after turning of sun hemp or dhaincha into the soil for green manuring or mix 1 kg of *Trichoderma* formulation in 100 kg of farmyard manure and cover it for 7 days with polythene. Sprinkle the heap with water intermittently. Turn the mixture in every 3-4 days interval and then broadcast in the field.
5. **Aerial spraying / Wound dressing:** *Trichoderma* has been successful applied to the aerial plant parts for the biocontrol of decay fungi in wounds on shrubs and trees.
6. **Plant Treatment:** Drench the soil near stem region with 10 g *Trichoderma* powder mixed in a liter of water.

Precaution while using *Trichoderma* as a bioagent

» Choose the appropriate *Trichoderma* strain that is specifically effective against the target pathogen or for the intended plant growth promotion. Different *Trichoderma* species and strains have varying levels of effectiveness against different pathogens.

- » Ensure that you are using a high-quality *Trichoderma* formulation from a reputable source. The product should have a viable and sufficient number of colony-forming units (CFUs) to be effective.
- » Avoid use of *Trichoderma* in extreme temperatures, excessively high or low pH levels, and harsh environmental conditions that could stress the fungus.
- » Don't use chemical pesticides after application of *Trichoderma* for 10-15 days.
- » If you plan to use *Trichoderma* with other agricultural inputs, conduct compatibility tests to ensure that there are no adverse interactions between them.
- » Don't use *Trichoderma* in dry soil because moisture is an essential factor for its growth and survivability.
- » Don't put the treated seeds in direct sun rays.
- » Don't keep the treated FYM for longer duration.
- » Applying excessive amounts of *Trichoderma* may not provide additional benefits and could lead to wastage and unnecessary expenses.
- » When handling *Trichoderma* formulations, wear appropriate protective clothing, such as gloves and a mask, to avoid direct contact with the product.
- » Regularly monitor the treated plants for signs of disease control or growth promotion. Evaluate the effectiveness of *Trichoderma* and adjust the application strategy if needed.

Conclusion

In conclusion, the mass production technology of *Trichoderma* has undergone significant advancements, transforming it from a laboratory curiosity to a commercially viable bioagent in agriculture. The use of liquid carrier media has emerged as a preferred method for large-scale production due to its scalability, homogeneity, and higher spore yield compared to solid-state fermentation. The development of affordable and readily available solid materials as substrate substitutes has addressed the scarcity and high cost of traditional synthetic solid media. Utilizing agricultural and food industry by-products such as sawdust, rice husk, and vegetable waste not only reduces production costs but also promotes sustainability by turning waste into valuable resources.

Trichoderma, with its diverse qualities, has shown great potential in agriculture. It can effectively reduce abiotic stresses, enhance physiological responses, and improve nutrient uptake in plants. Moreover, its role as an effective antagonist against plant

pathogens has led to successful biological control, reducing the reliance on chemical pesticides and promoting eco-friendly pest management.

Innovative formulations combining biocontrol and biofertilization capabilities have further enhanced *Trichoderma*'s efficacy, making it more potent and versatile against a wider range of diseases and promoting plant growth. However, the commercial application of *Trichoderma* also presents challenges, including associated costs and the need for strategic application. Ongoing research and development are essential to optimize production processes, improve formulation efficacy, and fine-tune application strategies to ensure cost-effectiveness and maximum impact on agricultural practices.

As we continue to explore the potential of *Trichoderma* and harness its beneficial properties, it holds great promise in contributing to sustainable agriculture, promoting food security, and mitigating the environmental impacts of conventional agricultural practices. By utilizing mass production technology effectively and responsibly, *Trichoderma* can play a pivotal role in shaping a more sustainable and resilient agricultural future.

Future Prospect

The future prospects for the mass production technology of Trichoderma are promising, driven by advancements in biotechnology, fermentation processes, and the growing demand for sustainable and eco-friendly agricultural solutions. Some potential areas of development include

1. **Strain selection and optimization**: Continued research on *Trichoderma* strains can lead to the discovery of new, more effective biocontrol agents and biofertilizers. Genetic and metabolic engineering may be employed to enhance specific traits, such as stress tolerance, nutrient utilization, and disease suppression capabilities.
2. **Bioprocess optimization**: Further optimization of liquid fermentation processes can improve spore yield, viability, and overall productivity. Advancements in bioreactor design and process control can lead to more efficient and cost-effective mass production of *Trichoderma.*
3. **Substrate utilization**: Research on alternative and renewable substrates for *Trichoderma* cultivation can help reduce production costs and reliance on limited resources. This may involve exploring waste materials from various industries or agricultural by-products as potential substrates.
4. **Encapsulation and formulation**: Developing innovative formulations and encapsulation techniques can enhance the shelf life and stability of *Trichoderma* products. These advancements can improve their viability during storage and transportation, expanding their accessibility to remote area**.**

5. **Biological nanotechnology**: Emerging technologies, such as nanotechnology, may open new possibilities for delivering *Trichoderma* and its bioactive compounds more efficiently to plants. Nano-formulations could improve the bioavailability and targeted delivery of *Trichoderma* for maximum efficacy.
6. **Integration with precision agriculture**: Integrating *Trichoderma*-based products with precision agriculture technologies, such as drones and sensors, can optimize application strategies based on real-time data. This can lead to precise and targeted treatments, reducing wastage and enhancing cost-effectiveness.
7. **Combined biocontrol approaches**: Combining Trichoderma with other beneficial microorganisms or biocontrol agents could create synergistic effects for improved disease suppression and overall plant health. Understanding the interactions between different beneficial microbes can lead to effective biocontrol consortia.
8. **Climate-smart solutions**: Research on Trichoderma strains adapted to specific climatic conditions can offer climate-smart solutions for regions facing environmental challenges, such as increased drought or salinity**.**
9. **Regulatory support**: Governments and regulatory bodies play a vital role in supporting the mass production and commercialization of *Trichoderma*-based products. Streamlined regulations, incentives, and subsidies can promote its adoption in agriculture.

As agriculture faces the challenges of feeding a growing global population while minimizing environmental impacts, the future of *Trichoderma* mass production technology holds immense potential. By combining scientific advancements with sustainable practices, *Trichoderma* can play a crucial role in shaping a more resilient and eco-friendly agricultural sector. Continued research, development, and collaborative efforts among scientists, policymakers, and industry stakeholders will drive the successful implementation of *Trichoderma*-based solutions in the years to come.

References

Batta, Y. A. (2005). Postharvest biological control of apple gray mold by *Trichoderma harzianum* formulated in an invert emulsion. *Crop Protection*, **23**(1): 19-26.

Connick, W., Daigle, D. and Quimby, P. (1991). An improved invert emulsion with high water retention for mycoherbicide delivery, *Weed Technology*, **5**: 442-444.

Fravel, D. R., Rhodes, D. J. and Larkin, R. P. (1999). Production and commercialization of biocontrol products. In: Integrated pest and disease management in greenhouse crops (Albajes, R., Lodovica Gullino, M., Van Lenteren, J. C. and Elad, Y. eds.), *Kluwer Academic Publishers*, Boston pp. 365-376.

Gupta, R., Saxena, R. K. and Goel, S. (1997). Short Communication: Photoinduced sporulation in *Trichoderma harzianum*–an experimental approach to primary events. *World Journal of Microbiology and Biotechnology,* **13**(2): 249-250.

Harman, G. E., Howell, C. R., Viterbo, A., Chet. I. and Lorito, M. (2004). *Trichoderma* sp. -opportunistic, avirulent plant symbionts. *Nature Reviews,* **2**: 43-56.

Howell, C. R. (2013). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases. The history and evolution of current concepts. *Plant diseases,* **87**(1): 5-9.

Jeyarajan, R. (2006). Prospects of indigenous mass production and formulation of *Trichroderma*, In current status of biological control of plant diseases using antagonistic organisms in India (Eds Rabindra RJ Ramanujam B), *Project Directorate of Biological Control*, Bangalore, pp. 74-80, 445.

Jeyarajan, R. and Nakkeeran, S. (2000). Exploitation of microorganisms and viruses as biocontrol agents for crop disease management. In: Biocontrol Potential and their Exploitation in Sustainable agriculture, (Ed. Upadhyay *et al.,*) *Kluwer Academic/Plenum Publishers*, USA pp. 95-116.

Jeyarajan, R., Ramakrishnan, G., Dinakaran, D. and Sridar, R. (1994). Development of products of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot diseases. In "Biotechnology in India" (Ed Dwivedi B.K.) *Bioved Research Society*, Allahabad. pp. 25-36.

Khan, S., Bagwan, N. B., Iqbal M. A. and Tamboli, R. R. (2011). Mass multiplication and shelf life of liquid fermented final product of *Trichoderma viride* in different formulations. *Advance in Bioresearch,* **2**(1): 178-182.

Lewis, J. A. (1991). Formulation and delivery system of biocontrol agents with emphasis on fungi Beltsville symposia. In: The rhizosphere and plant growth (Keister, D. L. and Cregan, P. B. eds.). *Agricultural Research*, **14**: 279-287.

Parkash, V. and Saikia, A. J. (2015). Production and multiplication of native compost fungal activator by using different substrates and its influence on growth and development of *Capsicum chinensis* Jacq. "Bhut Jolokia. *Int Biotechnol Research Article* ID 481363. DOI: 10.1155/2015/481363.

Sabalpara, A. N. (2014). Mass multiplication of biopesticides at farm level. *Journal of Mycology and Plant Pathology,* **44**(1): 1-5.

Sankar, P. and Jeyarajan, R. (1996). Biological control of sesamum root rot by seed treatment with *Trichoderma* spp. and *Bacillus subtilis*. *Indian Journal of Mycology and Plant Pathology,* **26**: 147-53.

Seethapathy, P., Kurusamy, R. and Kuppusamy, P. (2017). Soil borne diseases of major pulses and their biological management. *An Int J Agri.,* **2**(1): 1-11.

Sriram, S., Palanna, K. B. and Ramanujam, B. (2010). Effect of chitin on the shelf life of *Trichoderma harzianum* in talc formulation. *Indian Journal of Agriculture Sciences,* **80**: 930-932.

Sriram, S., Roopa, K. P. and Savitha, M. J. (2011). Extended shelf- life of liquid fermentation derived talc formulations of *Trichoderma harzianum* with the addition of glycerol. *Crop protection,* **30**: 1334-1339.

Subash, N., Viji, J., Sasikumar, C. and Meenakshisundaram, M. (2013). Isolation, media optimization and formulation of *Trichoderma harizanum* in agricultural soil. *J of Microbiol and Biotechnol.,* **3**(1): 61-64.

Swant, I. S. and Sawant, S. D. (1996). A simple method for achieving high cfu of *Trichoderma harzianum* on organic wastes for field applications. *Indian Phytopathology*, **9**: 185-87.

Woo, S. L., Ruocco, M., Vinale, F., Lombardi, N., Pascale, A., Lanzuise, S., Manganiello, G. and Lorito, M. (2014). *Trichoderma* based products and their wide spread use in agriculture. *The Open Mycology J.,* **8**: 71-126. DOI: 10.2174/1874437001408010071

10 Uncovering and Preserving Bio-Agents

Jyoti Kumari[1], Ankit Kumar Singh[2], Kumar Aditya[3] and Puja Kumari[4]

[1]Ph.D. Scholar, Deptt. of Plant Pathology, RAC, BAU, Kanke, Ranchi, JH
[2]Ph.D. Scholar, Deptt. of Entomology, RAC, BAU, Kanke, Ranchi, JH
[3]Ph.D. Scholar, Deptt. of Plant Pathology, IAS, BHU, Varanasi, U.P.
[4]Ph.D. Scholar, Deptt. of Plant Pathology, BAU, Sabour, Bihar

Abstract

Pathogens and bioagents that affect plant health are studied in plant pathology. In order to effectively control disease, conduct research, and promote conservation, it is essential for plant pathology to find and preserve bio-agents. Finding and identifying pathogens is the first step in the process of discovering bio-agents. For precise and quick detection, a variety of methods are used, including molecular diagnostics, serological assays, and microscopy. Identification entails employing genetic, morphological, or biochemical markers to characterize the bio-agents at the species or strain level. Researchers can better comprehend bio-agents' biology, pathogenicity, and interactions with host plants thanks to the knowledge they have learned about them. Discovering and following the distribution and dynamics of bio-agents depends heavily on surveillance and monitoring. In order to identify and track the appearance, dissemination, and effects of bioagents, these efforts need systematic and recurrent surveys of plants and agricultural systems. By facilitating the quick gathering, analysis, and interpretation of data, advanced technologies, such as remote sensing, geographic information systems (GIS), and big data analytics, improve surveillance and monitoring capabilities. Techniques for preservation are crucial for preserving the viability and stability of bioagents. For long-term storage and use of samples, a variety of preservation techniques are used, including drying, freezing, cryopreservation, and preservation in culture collections. By removing

water from pathogens and plant materials, drying stops microbial development and enzymatic activity.

Keywords: Bio-agents, Plant Pathology, Uncovering, Preserving, Detection, Identification, Surveillance, Monitoring, Preservation, Techniques.

Introduction

Biofactors, also called biological agents, play a key role in plant pathology as they comprise a wide variety of microorganisms and other biological entities that can cause plant diseases. Understanding biological agents is critical to effective disease management and sustainable agriculture (Agrios, 2005). Biofactors in plant pathology include bacteria, fungi, viruses, phytoplasma, viroids, nematodes and plant parasites. Each category of biological agents exhibits unique properties and mechanisms of infection that lead to different disease symptoms and plant health effects. For example, bacterial pathogens can cause wilt, necrosis, or canker, while fungal pathogens often cause leaf spot, downy mildew, or rot. Viruses can cause mosaic patterns, stunting, or deformity in infected plants, while nematodes can cause root thickening or cyst formation. Understanding the different types of biological agents is critical to accurately identifying and treating disease (Gaur *et al.,* 2021 and Gnanamanickam& Immanuel, 2006). Bio-agents have a significant impact on plant health, agricultural productivity and global food security. They can cause devastating diseases that result in crop losses, reduced crop quality, and higher productioncosts. Plant diseases caused by biological agents can also result in trade barriers that limit the internationalmovement of agricultural products. In addition, biological agents can rapidly evolve, adapt to new hosts, and overcome resistance, posing an ongoing challenge to disease control efforts.

Through the creation of poisons or by entering plant tissues and upsetting normal physiological processes, bioagents can directly harm plants. They may also weaken plants, leaving them more vulnerable to secondary infections or environmental challenges, which could have an indirect impact on plant health. Additionally, some bio-agents can affect how plants grow and develop, leading to anomalies or malformations that reduce crop output in general.

Effective disease management depends on the accurate detection and identification of bio-agents. Microscopic examination, serological assays, molecular methods (such polymerase chain reaction and DNA sequencing), and bioassays are some of the methods used for this aim. These techniques enable scientists and plant pathologists to pinpoint the precise bio-agent responsible for the illness, allowing for the implementation of focused control measures.

Techniques for identification and detection

Techniques for identification and detection are essential in plant pathology for making an accurate diagnosis of plant diseases and locating the accountable bio-agents. Effective disease management tactics have substantially benefited from the development of these tools. An overview of the many identification and detection methods used in plant pathology, including microscopic analysis, serological assays, molecular methods, and bioassays, is provided in this article. In order to identify and detect bio-agents and enable focused disease control actions, each technique performs a specific function.

» **Microscopic Examination:** For preliminary observations and the identification of bio-agents, microscopic inspection continues to be a crucial technique in plant pathology. This method entails using light or electron microscopes to see the morphological features of bio-agents like worms, bacterial cells, and fungal spores (Clark & Adams, 1977). Key characteristics, such as spore shape, the presence of conidiophores, or nematode stylet structure, can be identified through microscopic analysis. Additionally, staining methods can improve visibility and help with identification, such as lactophenol cotton blue. For quick preliminary identification of bio-agents in lab and field settings, microscopy is a flexible and affordable approach.

» **Serological Assays:** Immunological methods known as serological assays are used to identify certain antigens or antibodies linked to plant pathogens. The serological procedures immunoblotting (sometimes called Western blotting) and enzyme-linked immunosorbent assay (ELISA) are frequently used in plant pathology. These tests make use of antibodies that firmly attach to the target antigens, identifying and measuring the bio-agents. For the quick detection of bacterial, viral, and fungal diseases, serological tests are beneficial. They are especially helpful when a high sample throughput is required. They are commonly used in large-scale pathogen screening programmes and regular plant disease diagnostics.

» **Molecular Techniques:** Through the development of extremely sensitive and focused methods for identifying and detecting bio-agents, molecular approaches have revolutionised plant pathology. The widely used molecular technique known as polymerase chain reaction (PCR) amplifies particular DNA sequences from the genomes of bio-agents. Pathogens can be quickly and precisely identified using PCR-based techniques such as multiplex PCR, real-time PCR (qPCR), and conventional PCR (Dasgupta, 2014). Additionally, bio-agents can have their entire genetic makeup determined using

DNA sequencing techniques like Sanger sequencing and next-generation sequencing (NGS), which helps with identification and characterization. Additionally, molecular methods make it easier to find genetically unique strains or variants within a population of bioagents as well as to detect low-level infections (Mullis &Faloona, 1987).

» **Bioassays:**In order to find the presence of bio-agents or assess their pathogenicity, bioassays use living creatures like insect vectors or indicator plants. Following immunisation, indicator plants that are vulnerable to particular bioagents are employed to monitor illness symptoms or signs. One well-known application of a bioassay is the use of tobacco (Nicotiana tabacum) as an indicator plant for the detection of the Tobacco mosaic virus. Insects like aphids or leafhoppers are used in insect vector-based bioassays to distribute and transmit bioagents. These bioassays are useful for identifying viruses and phytoplasmas that are spread via insect vectors. Under controlled circumstances, bioassays offer useful information on pathogen infectivity, host range, and disease course.

» **Integration of Techniques:**To maximise accuracy and reliability, various identification and detection approaches are frequently combined in practise. A thorough assessment of the bio-agents present in a given sample is made possible by the combination of microscopic investigation, serological assays, and molecular approaches. The presence of particular antigens may be confirmed by serological assays, which are then followed by molecular techniques to identify the specific bio-agent at the genetic level. For instance, initial microscopic examination may disclose certain morphological traits. This multidisciplinary approach improves diagnostic precision, particularly when several bioagents are present in a sample or when they show morphological similarities.

Sample Collection and Preservation in Plant Pathology

To correctly diagnose and identify plant diseases and the associated bioagents, sample collection and preservation are essential processes in plant pathology. The gathered samples are kept intact during transportation and storage thanks to appropriate collection procedures and efficient preservation processes, enabling accurate laboratory analysis. In order to achieve the best sample quality and precise disease diagnosis, this article offers an overview of sample collecting and preservation techniques in plant pathology, including recommendations for collection, handling, and storage.

Sample Collection Guidelines:

For representative plant material that truly reflects the disease condition, proper sample collection is crucial. When collecting samples, the following rules should be followed:

1. Selected Sampling locations: Pick plants exhibiting typical disease symptoms as well as other sampling locations that represent the afflicted area. In addition, it's crucial to get samples from various plant sections, such as leaves, stems, fruits, or roots, depending on the symptoms seen.

2. Sample Size: Gather a sufficient sample size to ensure there is enough data for analysis. Depending on the sort of study needed, the sample size may change, however it is typically advised to gather multiple samples from various plants within the affected area.

3. Sampling Instruments: To harvest plant samples, use clean, sterile instruments such scissors, knives, or pruners. Between sampling various plants, sterilise the instruments to avoid cross-contamination.

4. Sample Packaging: To avoid contamination, moisture loss, or desiccation, place the gathered plant samples in clear, labelled, and sealed plastic bags or containers. It could be required to incorporate absorbent material in the packaging for samples with high moisture content, such as fruits or tissues with high water content, to limit excess moisture.

5. Documentation: Note pertinent details about the sample, such as the site, the date, the type of plant it came from, its developmental stage, and any symptoms that were noticed. Accurate diagnosis and subsequent research or extension activities are made possible by this documentation.

Sample Handling and Transportation:

Proper handling and transportation of obtained samples are critical to maintain sample quality throughout transit to the laboratory. The following considerations should be taken into account:

1. Sample handling precautions: Handle samples gently to reduce contamination or mechanical damage. Avoid coming into contact with healthy plants or any other potential contaminating elements.

2. Temperature Control: Preserve proper temperatures while transporting samples. To avoid degradation or changes in sample properties, cool or refrigerate samples if necessary, particularly when delays in shipping are anticipated.

3. Rapid Transportation: Hasten the delivery of samples to the lab to reduce sample

quality loss. Deliver samples as soon as feasible after collection, especially if they are living or likely to degrade quickly.

Sample Preservation Methods

To maintain their original properties and stop the deterioration of pathogens or biochemical components, gathered plant samples must be preserved. The type of sample and the particular analysis to be performed determine the preservation method to be used. In plant pathology, frequent preservation techniques include:

1. Refrigeration: Most plant samples can be preserved for a brief period of time by refrigeration at temperatures between 2 and 8 °C. The sample quality is maintained for a few days to a few weeks thanks to this method's ability to slow down metabolic processes and inhibit the growth and activity of infections.

2. Freezing: For long-term preservation of plant samples, freezing is a successful technique. Depending on the precise requirements, samples can be frozen at -20°C or -80°C. The metabolic processes and pathogen growth are stopped by freezing, which prolongs the storage time of samples—sometimes even years.

3. Drying: Herbarium specimens, fruits, seeds, and other materials with a high moisture content can be preserved by drying. Depending on the type of sample and the required analysis, many drying techniques can be used, including air drying, oven drying, and freeze drying.

4. Chemical Fixatives: Plant samples are preserved using chemical fixatives like ethanol, formalin, or glycerol so they may be examined under a microscope or have their DNA examined. Fixatives maintain cellular structures and stop cell morphology from deteriorating or changing. The specific analysis and the sample's properties will determine the fixative to use.

Disease Surveillance and Monitoring in Plant Pathology

In order to understand the occurrence, distribution, and dynamics of plant diseases and the bioagents that cause them, it is essential to conduct disease surveillance and monitoring. Early identification, prompt action, and well-informed decision-making for disease management strategies are made possible by efficient surveillance programmes. This article gives a general overview of plant pathology's disease surveillance and monitoring methods, including field research, remote sensing, and molecular diagnostic equipment.

» **Field Surveys:**In order to evaluate the existence, severity, and distribution of plant diseases, field surveys, which involve systematic observations and sampling, are a crucial part of disease monitoring. In order to locate disease

hotspots, track the spread of the illness, and calculate the incidence and prevalence of the disease, plant pathologists and researchers perform field surveys (Mundt, 2002). Various techniques are used during field surveys, such as visual observations, symptom evaluations, and sample gathering. These studies can be carried out through systematic transects, random sampling, or focused studies based on historical disease data or recognised risk factors. For illness monitoring, trend analysis, and early warning systems, field surveys offer useful data.

» **Remote Sensing:** Various sensors and imaging systems are used in remote sensing techniques to identify and track plant diseases. Spectral data from plants is captured and analysed by remote sensing platforms, including satellites, flying drones, or ground-based sensors, to reveal the existence, severity, or stress of diseases. In plant pathology, multispectral, hyperspectral, and thermal imaging are frequently utilised remote sensing methods. Remote sensing can assist in identifying disease-related changes in plant physiology, canopy reflectance, or temperature by examining particular wavelengths or thermal trends. In order to detect and track disease outbreaks early on, remote sensing offers a speedy and extensive method of disease surveillance.

» **Molecular Diagnostic Tools:** By enabling quick and precise bio-agent detection in plant samples, molecular diagnostic technologies have revolutionised disease surveillance and monitoring in plant pathology. Pathogens can be detected and identified based on their unique nucleic acid sequences using molecular methods including quantitative PCR (qPCR), loop-mediated isothermal amplification (LAMP), and polymerase chain reaction (PCR). With the use of these methods, pathogens at low concentrations in plant tissues can be found, and the results are extremely sensitive and precise. Additionally, complex microbial communities linked to plant diseases can be recognised and characterised thanks to genomic approaches like metagenomics and next-generation sequencing (NGS). Early detection, accurate identification, and monitoring of bio-agents in plants are made possible by molecular diagnostic techniques, allowing for quick responses and focused disease control measures.

» **Data Management and Analysis:** Large volumes of data are produced by disease surveillance and monitoring, which call for efficient management and analysis. Data management and geographic information systems (GIS) aid in the organisation and visualisation of disease data, allowing for the spatial and temporal investigation of disease patterns. GIS enables the mapping of illness occurrences, the detection of disease clusters, and the evaluation of

the migration and spread of diseases. The interpretation and modelling of disease data is made easier by the use of sophisticated statistical techniques such spatial analysis, trend analysis, and time-series analysis. For thorough disease surveillance and monitoring programmes, data integration, sharing, and cooperation among plant pathologists, researchers, and stakeholders are essential.

» **Early Warning Systems:** The creation of early warning systems, which aim to give farmers, decision-makers, and stakeholders timely alerts and actionable information, is aided by disease surveillance and monitoring. Early warning systems assess disease risk and probable outbreaks by combining disease data with weather data, crop growth models, and predictive algorithms. Early warning systems can assist in the implementation of timely treatments, such as targeted pesticide applications, cultural practises, or resistant types, to reduce the effect of diseases by combining data from surveillance programmes and using prediction models. These solutions promote overall crop health, decrease economic losses, and improve disease management decision-making.

Biosecurity Measures in Plant Pathology

In plant pathology, biosecurity precautions are essential for stopping the introduction, establishment, and spread of hazardous bioagents that can cause deadly plant diseases. In order to safeguard agricultural systems, maintain plant health, and guarantee food security, effective biosecurity policies and practises are essential (Haque & Khan, 2021). An overview of plant pathology's biosecurity measures, such as containment facilities, surveillance systems, and legal frameworks, is given in this article. Plant pathologists and other interested parties can reduce the dangers associated with bioagents and protect plant health by putting in place strict biosecurity measures.

» **Quarantine Protocols:** Fundamental biosecurity procedures including quarantine protocols are employed to prevent the cross-border movement of plant materials that could harbour bioagents. Regulations for quarantine are designed to stop the spread of invasive illnesses, pests, or weeds. To maintain compliance with phytosanitary requirements, plant material, whether seeds, cuttings, or live plants, is submitted to examination and certification (CABI, 2020). Facilities for quarantine, such plant inspection stations or border crossings, have qualified staff and diagnostic equipment to find and halt any hazardous bioagents. The first line of defence against the spread of novel plant diseases is quarantine procedures.

» **Containment Facilities:** Specialised research or diagnostic labs known as containment facilities are created to handle and analyse high-risk bioagents

in a confined setting. To avoid the unintentional release of bioagents, these facilities adhere to stringent safety and containment standards that safeguard researchers, staff members, and the surrounding area. Physical barriers, such as biosafety cabinets, air filtration systems, and controlled access controls, are installed in containment facilities (FAO, 2021). Bio-agents are handled securely and safely within these facilities thanks to appropriate training, protocols, and standard operating procedures. Research, the creation of diagnostics, and the management of disease all require containment facilities in order to investigate and comprehend high-risk bio-agents.

» **Surveillance Programs:**Programmes for surveillance are crucial parts of biosecurity measures because they make it possible to identify and track bioagents in agricultural systems early on. Monitoring for the presence of bioagents in crops, plant populations, or certain geographic areas is part of surveillance. In order to conduct surveillance operations including visual inspections, symptom monitoring, and pathogen detection procedures, plant pathologists, researchers, and regulatory agencies work together (APHIS, 2021). Programmes for surveillance offer vital information on the distribution, occurrence, and changes in populations of bio-agents, enabling early intervention and disease management tactics.

» **Regulatory Frameworks:**Regulatory frameworks serve as the foundation for biosecurity measures by outlining the rules, regulations, and policies that must be followed to stop the introduction and spread of dangerous bioagents. Regulatory organisations like national plant protection organisations create and uphold phytosanitary standards and rules. The import and export of plant materials, plant health certification, and pest risk analyses may all be subject to these restrictions (EPPO, 2021). The creation of pest-free zones, the use of biological control agents, and the registration and use of pesticides are all tactics for managing pests and diseases that can be made easier by regulatory frameworks. Implementing consistent and efficient biosecurity measures is ensured by adherence to regulatory frameworks.

» **Training and Education:**Plant pathologists, researchers, producers, and other stakeholders must be made more aware of and understand biosecurity measures through training and education programmes. Programmes for training participants cover biosecurity threats, best practises, and the significance of adhering to protocol (Pautasso*et al.,* 2015). The adoption of biosecurity measures, such as sound agricultural practises, integrated pest control, and early disease detection, is encouraged via educational efforts. Training and education programmes support the adoption of responsible

behaviours and the growth of a watchful and proactive attitude to plant health protection by building a culture of biosecurity awareness.

» **International Collaboration and Cooperation:**In a globalised world, cooperation and international collaboration are essential for effective biosecurity measures. Plant pathologists, researchers, and regulatory organisations work together internationally to share knowledge, skills, and resources (Subasinghe& Shinn, 2023). In order to handle new biosecurity concerns, collaborative projects put a focus on creating global standards, harmonising laws, and conducting joint research. The ability of nations and regions to exchange information and experiences improves the global community's ability to manage and prevent plant diseases.

Biological Control in Plant Pathology

Plant pathology uses biological control as a sustainable and environmentally friendly method of managing plant diseases brought on by bio-agents (Fravel, 2005). Plant pathogen populations can be suppressed or reduced through the use of beneficial microbes, predators, parasites, or other species. The ideas, mechanisms, and illustrations of effective biological control methods are covered in this article's overview of biological control in plant pathology (Harman*et al.,* 2004). Biological control offers efficient and sustainable alternatives to traditional disease management techniques by utilising nature's own control agents.

Principles of Biological Control:

Biological control relies on three fundamental principles:

1. Conservation: Effective biological control depends on the preservation of natural adversaries. Plant pathogens are naturally suppressed by natural enemies such as advantageous microbes, parasitic wasps, or predatory insects who attack, parasitize, or engage in conflict with them (Van Lenteren*et al.,* 2018). Their numbers are maintained in agricultural settings by conservation techniques include creating adequate habitats, cutting back on pesticide use, or applying social norms that support natural enemies.

2. Augmentation: In augmentation biological control, natural enemies are produced in large quantities and released into the environment to increase their population and control of plant infections. To combat particular plant infections, commercially generated natural enemies can be applied in the field, such as advantageous nematodes or entomopathogenic fungi (Rumbos &Athanassiou, 2017). Augmentation is particularly useful when the natural enemy population is insufficient or when additional control is required to manage severe disease outbreaks.

3. Manipulation: In order to encourage natural enemies and strengthen their influence on plant diseases, manipulative biological control entails altering the environment or agricultural practises. Crop rotation, intercropping, or the use of cover crops are examples of manipulative techniques that can be used to generate diversified habitats that encourage the presence and activity of beneficial species (Lacey*et al.*,2001). Plant diseases' life cycles are intended to be disrupted, and conditions that inhibit their growth and spread are intended to be created.

In order to inhibit plant diseases, biological control agents use a variety of techniques.

1. Competition: Plant pathogens can be restricted in their growth and colonisation by beneficial bacteria, which can outcompete them for nutrients and space. Nutrient competition, occupying space, or the creation of chemicals that limit pathogen growth are a few examples of competitive mechanisms.

2. Antibiosis: Biological control agents can create antimicrobial substances, such as antibiotics or secondary metabolites, that obstruct plant pathogens' ability to grow or function. These substances prevent infections from performing crucial physiological functions, which reduces their virulence or pathogenicity.

3. Parasitism and Predation: Plant pathogens can be attacked and eaten by parasitic or predatory organisms. Nematodes and ladybirds are examples of predators that consume nematodes or plant-pathogenic insects to control their populations and stop the spread of illness (Boomsma*et al.,* 2014). Plant pathogens are infected and killed by parasitic organisms like parasitic fungus and parasitic wasps, which restricts their ability to spread and harm plants.

Examples of Biological Control in Plant Pathology: Several instances show how effectively biological control is used in plant pathology:

1. *Trichoderma* spp.: A number of *Trichoderma* species are frequently employed as biocontrol agents against numerous plant diseases(Harman *et al.,* 2004). These fungi form symbiotic relationships with plant roots and generate enzymes that break down pathogens' cell walls, preventing infection. *Trichoderma* spp. can improve plant growth and disease resistance while being effective against soilborne pathogens like *Fusarium* or *Rhizoctonia*.

2. *Bacillus subtilis*: This helpful bacterium is employed in biological pest management. It produces antimicrobial substances, like as enzymes and antibiotics, that prevent the growth of bacterial and fungal infections. Numerous bacterial and fungal diseases, as well as other plant pathogens, are successfully combated by *Bacillus subtilis*.

3. *Aphidius* species: Parasitic wasps of the genus *Aphidius* are employed to biologically

control aphids, which are capable of spreading plant viruses. By implanting eggs into the bodies of aphids, these wasps parasitize them, causing the aphids to perish. *Aphidius* spp. aid in the regulation of viral transmission and the avoidance of crop damage by lowering aphid populations.

4. *Beauveria bassiana*: This entomopathogenic fungus is used to manage insect pests biologically. Insects with this fungus, such as whiteflies or thrips, develop illness symptoms that ultimately result in their demise. The plant *Beauveria bassiana* has the ability to control a variety of insect pests, lowering their numbers and averting agricultural loss.

5. *Phytoseiulus persimilis*: A predatory mite employed for biological control of spider mites, an important problem in many crops, is *Phytoseiulus persimilis*.

Preservation Techniques in Plant Pathology

By maintaining the long-term viability and stability of plant materials, pathogens, and other bio-agents, preservation techniques serve a critical role in plant pathology. Maintaining the integrity and viability of samples is crucial for enabling precise diagnostics, in-depth study, and long-term storage. An overview of preservation methods used frequently in plant pathology, including as drying, freezing, cryopreservation, and preservation in culture collections, is given in this article. The management and investigation of plant diseases require a thorough understanding of and application of proper preservation procedures.

- **Drying:**Drying, a common preservation technique, involves eliminating water from pathogens and plant materials in order to prevent microbial development and enzymatic activity. Depending on the sample type and intended results, different drying processes might be used. A quick and affordable technique for preserving leaves, flowers, and other plant parts is air drying. In this technique, materials are dried by air in a drying chamber or at ambient temperature with typically low humidity. Alternately, oven drying can be used to dry items more quickly and at a set temperature. Freeze-drying, also known as lyophilization, is the process of rapidly eliminating water from materials after they have been frozen. This method preserves cellular structures and biological components better than other methods. Plant samples are frequently dried in order to preserve them for long-term storage, DNA extraction, and herbarium collections.
- **Freezing:**A preservation method called freezing entails keeping samples at extremely low temperatures, usually below -20°C or even -80°C, depending on the needs of the sample. The integrity and viability of plant materials

and pathogens are efficiently maintained by freezing, which slows down metabolic processes and enzyme activity. For the preservation of plant tissues, diseases, and insect vectors, freezing is frequently used. Cryopreservation methods for plant tissues, such as vitrification or encapsulation-dehydration, are frequently utilised to guarantee the material's long-term viability and genetic stability. In research labs, seed banks, and culture collections, freezing is frequently used for long-term sample storage and retrieval.

» **Cryopreservation:**An sophisticated preservation method called cryopreservation includes storing biological components including cells, tissues, or embryos at extremely low temperatures, such as liquid nitrogen (-196°C) (Benson, 2008). To avoid the development of harmful ice crystals during the freezing process, cryopreservation uses cryoprotective chemicals and controlled cooling and warming rates (Reed, 2008). This method enables the long-term viability and genetic integrity preservation of plant cells, tissues, and genetic resources, such as seeds, pollen, or embryos. Maintaining diversified germplasm collections and genetic resources, as well as preserving uncommon, endangered, or resistant plant species, calls for cryopreservation (Ye, 2012).

Preservation in Culture Collections

Living bio-agents can be preserved in culture collections, such as microbial or plant tissue culture collections, for long-term storage and upkeep. Plant tissue cultures, fungal and bacterial strains, and harmful and helpful microbes are all stored in culture collections. Agar slants, liquid media, or freeze-dried ampoules are used to store microbial strains as pure cultures, whereas solid or liquid media combined with the right nutrients and growth regulators are used to retain plant tissue cultures. To guarantee the viability, purity, and genetic stability of conserved strains or cultures, culture collections adhere to stringent protocols and quality control measures. The availability, exchange, and distribution of bio-agents for study, diagnosis, and applications in plant pathology are made possible by preservation in culture collections.

Other Preservation Methods

1. Chemical preservation: To stabilise cellular structures, stop decay, and retain sample integrity, some plant samples or bio-agents can be kept in chemicals like formalin, ethanol, or glycerol. For applications such as DNA extraction, histology, or microscopy, chemical preservation is frequently used.

2. Preservation in desiccants: Samples can be preserved by being dried out using desiccants like silica gel or molecular sieves, which stop microbial development

and deterioration. For the preservation of seeds, pollen, or other plant reproductive resources, desiccants are frequently used.

3. Preservation in specialised medium: Some microorganisms, such picky bacteria or plant pathogens, need to be preserved in specialised media. To sustain the viability and growth of the preserved organisms, these media contain specialised nutrients, growth agents, or antibiotics.

Pathogen Evolution and Adaptation

The genetic make-up, pathogenicity, and capacity to get past host defences of pathogens are all continually changing as a result of their adaptation to their host plants. Genetic diversity, natural selection, interactions with the host, and environmental factors all play active roles in the dynamic processes of pathogen evolution and adaptability. Effective disease control techniques require a thorough understanding of how pathogens change and adapt over time. The mechanisms, factors that affect adaptation, and implications for plant pathology are all covered in this article's summary of the evolution and adaptation of pathogens.

Mechanisms of Pathogen Evolution

Pathogens evolve through several mechanisms that generate genetic diversity and drive adaptive changes:

1. Mutation: In pathogens, mutation is the main source of genetic variation. DNA replication can result in spontaneous mutations, such as point mutations, insertions, deletions, or chromosomal rearrangements, which can alter the structure or function of a gene or a protein (Wolfe, 1985). Pathogens differ in their mutation rates, and RNA viruses have high mutation rates because their RNA replication mechanism is prone to errors.

2. Recombination: Recombination is the interchange of genetic material across several disease strains or species. Recombination can result in the acquisition of new genetic features such host resistance genes, virulence factors, or resistance to fungicides. The horizontal gene transfer that occurs frequently in bacteria and fungus is another factor in genetic diversity and adaptation.

3. Selection Pressure: Pathogen evolution and adaptation are driven by natural selection. Selection forces favour the survival and reproduction of individuals with favourable features, such as host resistance, the use of fungicides, or changes in the environment. Pathogens with a selective advantage can survive and proliferate by overcoming host defences or resisting chemical treatments.

Factors Influencing Pathogen Adaptation

Pathogen adaptation is influenced by various factors, including:

1. Host-Pathogen Interactions: Pathogen evolution is heavily influenced by how pathogens interact with the plants that serve as their hosts (Thrall, 2017). Pathogens are subject to selective pressure from host defences such as physical barriers, chemical substances, or immunological responses. It is more likely for pathogens to persist and spread disease if they can avoid or suppress the host's defences.

2. Genetic variety: Adaptation is made possible by genetic variety among populations of pathogens. The possibility of favourable features emerging through mutation or recombination increases with genetic diversity. Population size, migration, sexual reproduction, or clonal proliferation can all have an impact on genetic variety.

3. Environmental Factors: The evolution and adaption of pathogens can be influenced by environmental factors such as temperature, humidity, or the availability of nutrients. In response to environmental signals, pathogens may undergo phenotypic changes that allow them to flourish in particular environments or adapt to environmental challenges.

Understanding pathogen evolution and adaptation has important consequences for plant pathology, including the following.

1. Disease management: Strategies for managing diseases face difficulties due to pathogen development and adaptation. The effectiveness of chemical control techniques may be compromised by the rapid emergence of pesticide or fungicide resistance (Stukenbrock, 2012). Similar to how infections can break down host resistance by genetically altering host resistance genes. The creation of more long-lasting and efficient disease control measures can benefit from an understanding of the mechanisms and variables promoting pathogen adaptability.

2. Disease Spread and Emergence: As a result of pathogen evolution, new disease strains may appear or pathogen ranges may extend into new geographical regions (Jones, 2016). Plant health and agricultural productivity may be significantly impacted by the introduction of novel strains or variations. Monitoring and researching the development of pathogens can help in early disease identification, rapid response, and disease prevention.

3. Host-Pathogen Coevolution: Pathogens and the plants they infect are constantly engaged in a coevolutionary arms race (Barrett, 2009). In response to diseases evolving to defeat host defences, hosts also create novel forms of resistance. The results of host-pathogen interactions are influenced by this coevolutionary dynamic, which induces genetic alterations in both pathogens and hosts. Breeding strategies for

creating resistant crop types can be informed by an understanding of coevolutionary processes.

4. Molecular Diagnostics: Understanding the evolution of pathogens can help with the creation and application of molecular diagnostic techniques (Fisher, 2012). The creation of precise and accurate diagnostic assays can benefit from the identification of conserved or quickly evolving areas in the genomes of pathogens. The establishment of novel variations or strains with different virulence or resistance profiles can also be followed by tracking genetic changes in pathogen populations.

Emerging Bio-Agents in Plant Pathology

Emerging bio-agents are freshly discovered or recently evolved organisms that have the potential to cause serious illnesses in plants, which present considerable challenges to plant health and agriculture. These bio-agents can be viruses, pests, or invasive species that have just appeared on the scene or recently expanded their geographic range, which can have unexpected and unique effects on plant ecosystems (Chakraborty, 2016). The new bio-agents in plant pathology are discussed in this article along with their properties, the causes that contributed to their emergence, and the effects they may have on agricultural systems and plant health. For early diagnosis, quick response, and the creation of efficient management plans, an understanding of developing bio-agents is essential.

Characteristics of Emerging Bio-Agents

Emerging bio-agents in plant pathology possess several distinct characteristics:

1. Novelty: Emerging bio-agents are recently discovered or discovered organisms that had little or no effect on plant health in the past. They could be completely new species or strains, or they could be already-existing organisms that have expanded their distribution or acquired new virulence features (Garrett, 2011). They could also have overcome host resistance.

2. Rapid Spread: Emerging bioagents frequently possess the capacity for rapid dissemination and establishment in novel habitats. Globalisation, greater trade internationally, population movement, and climate change are among factors that may hasten the spread of bioagents to new areas or habitats.

3. Genetic Diversity: Emerging bioagents frequently have significant levels of genetic diversity, which enables them to adapt and get past host defences or environmental obstacles. Through mutation, recombination, or horizontal gene transfer, this genetic variety develops, allowing the bio-agents to quickly change and adapt to new circumstances.

Factors Influencing the Emergence of Bio-Agents

The development of bio-agents in plant pathology is influenced by a number of factors, including:

1. Globalisation and Trade: Unintentional cross-border migration of viruses, pests, and invasive species has been made easier by international trade and globalization (Pautasso, 2012). By accident introducing bio-agents into new environments, contaminated soil, infected host organisms, or sick plant material can spread disease.

2. Environmental Changes: Environmental changes, such as those brought on by climate change, changes in how land is used, or the destruction of habitats, might foster the creation of bioagents (Jones, 2009). The distribution, quantity, and activity of bio-agents can be impacted by changes in temperature, precipitation patterns, or the availability of suitable hosts, which might result in their emergence or worsening.

3. Evolution and Adaptation: Insects and pathogens can quickly evolve and adapt to defeat host defences, acquire pesticide resistance, or take advantage of new ecological niches (Pimentel, 2005). New features can be conferred via genetic modifications like mutations or recombination events, and they can also make it possible for bioagents to infect previously resistant host species.

Consequences of Emerging Bio-Agents: The development of bio-agents has important repercussions for agricultural systems and plant health.

1. Crop Losses: By infecting previously healthy plants or defeating current management techniques, emerging bio-agents might result in significant crop losses and economic harm. New viruses or pests can cause severe epidemics and the devastation of entire crops, affecting food security and way of life.

2. Ecological Disruption: Emerging bioagents have the potential to upset the ecological balance and local biodiversity. Native plants may be outcompeted by invasive species, ecosystem dynamics may be changed, or native flora and animals may begin to decline. Natural ecosystems can be disrupted by imbalances in the interactions between plants, pests, and predators, which can have a domino effect on ecosystem functioning.

3. Effects on Human Health: Some developing bioagents can be harmful to people's health. Sometimes, pathogens that affect plants can infect people directly or indirectly via contaminated foods, water supplies, or vectors. Zoonotic diseases, such as the spread of plant infections by ticks or insects, are examples.

4. Management Obstacles Emerging bioagents complicate disease management plans: Effective disease management may be hampered by the lack of prior knowledge and the restricted control options for newly discovered or emerging diseases.

Emerging bioagent control requires quick discovery, surveillance, the creation of resistant cultivars, or integrated pest management strategies.

5. Regulatory Considerations: To stop the introduction and spread of new bio-agents, rules and phytosanitary measures frequently need to be established as they emerge. Through quarantine measures, trade restrictions, and the deployment of control programmes, regulatory authorities play a critical role in monitoring and responding to new bio-agents.

Intellectual Property Rights in Plant Pathology

In many sectors, including plant pathology, intellectual property rights (IPRs) are essential for fostering and preserving innovation and creativity (Barton, 2005). IPRs offer legal frameworks to protect the rights of people or organisations who create new innovations, technologies, or plant types. IPRs are crucial in the context of plant pathology for promoting research and development, promoting information sharing, and facilitating the commercialization of plant disease management techniques (Chiarolla, 2013). An overview of intellectual property rights in plant pathology, including patents, rights of plant breeders, and other types of protection, is given in this article. In order to balance innovation, information access, and the fair distribution of benefits in the field of plant pathology, it is essential to understand IPRs.

Types of Intellectual Property Rights

There are several types of intellectual property rights relevant to plant pathology:

1. Patents: In exchange for disclosing their innovation to the public, inventors receive exclusive rights for a set time, usually 20 years. Patents for novel plant disease management techniques, diagnostic equipment, or genetically altered organisms may be granted in the field of plant pathology (Halewood, 2013). As they grant a legal monopoly on the commercial exploitation of the patented idea, patents safeguard the rights of inventors and promote investment in research and development.

2. Plant Breeders' Rights (PBR): PBR is a type of intellectual property protection intended exclusively for novel plant species (Gould, 2016). For a limited time, often 20 to 25 years, PBR allows breeders the only right to commercially exploit their newly created plant varieties. Plant breeders are incentivized by this protection to spend money creating enhanced cultivars with desirable attributes, such as disease resistance, yield potential, or quality traits.

3. Trade secret: Confidential and exclusive information that gives its owner a competitive advantage is referred to as a trade secret. Trade secrets in plant pathology may include private information on approaches to managing diseases, product formulations, or secret

procedures (Krattiger, 2007). Trade secrets can be protected permanently as long as the information is kept secret, unlike patents or PBR, which both require registration.

4. Copyright: Authors are granted exclusive rights to their original creations, such as books, scholarly articles, or software, under copyright protection. In plant pathology, written materials, software programmes, or educational resources pertaining to plant diseases, their management, or research discoveries are protected by copyright laws. The promotion of copyright protection helps spread knowledge while guaranteeing that authors are paid for and recognised for their creative efforts.

Benefits and Challenges of Intellectual Property Rights

Intellectual property rights offer various benefits and present specific challenges in the field of plant pathology:

1. Promoting Innovation: IPRs encourage firms, breeders, and academics to spend money on plant pathology study and development (Oh, 2018). IPRs provide innovators and breeders with the exclusivity they need to recoup their efforts and profit from their creations, which promotes further invention and technological development.

2. Technology transmit and Licencing: IPRs help researchers and breeders transmit technology and information to commercial companies. Innovative plant disease control techniques can be shared and made commercially viable through licencing agreements, which promotes their widespread acceptance and application.

3. Access to Genetic Resources: Plant genetic resources might be difficult to obtain and use because of intellectual property restrictions. Access to genetic resources for research or breeding objectives may be hampered by patent protection for plant genes or characteristics (Pal, 2018). Fair and sustainable plant breeding initiatives depend on striking a balance between the requirement for equitable access to genetic resources and the preservation of intellectual property rights.

4. Ethical Considerations: The equitable sharing of gains resulting from advances in plant pathology raises ethical questions when intellectual property rights are in play. To prevent disproportionate limits on access to plant disease control technology and knowledge, the effects of IPRs on small-scale farmers, developing nations, or public research institutes must be carefully assessed.

5. Collaboration and Technology Sharing: In terms of plant pathology, IPRs can either help or hurt collaboration and technology sharing. Agreements on intellectual property rights may be necessary for collaborative research projects in order to ensure that all parties involved receive the correct acknowledgment and benefits (Sharma, 2018). For collaboration and intellectual property protection to coexist and progress science, there must be a balance.

Conclusion

Identifying and protecting bio-agents are crucial components of plant pathology that support successful disease control, investigation, and conservation initiatives. Pathogens must be found and identified using a variety of methods, including molecular diagnostics, serological assays, and microscopy, in the process of locating bio-agents. Researchers can better understand the biology, pathogenicity, and interactions of bioagents by identifying them at the species or strain level, which helps them develop disease control methods.Discovering and following the distribution and dynamics of bio-agents depend heavily on surveillance and monitoring. The occurrence, dissemination, and effects of bio-agents are identified and tracked by routine surveys and monitoring efforts. Modern technologies like remote sensing, GIS, and big data analytics improve surveillance capabilities by enabling quick data collection, processing and interpretation of data. This information is crucial for early detection, prompt response, and implementation of appropriate control measures.

Techniques for preservation are crucial for preserving the viability and stability of bioagents. For long-term storage and use of samples, a variety of preservation techniques are used, such as drying, freezing, cryopreservation, and preservation in culture collections. By removing water from pathogens and plant materials, drying prevents microbial development and enzymatic activity. By maintaining materials at extremely low temperatures, freezing and cryopreservation procedures slow down metabolic processes. Plant pathology research and applications are made easier by the maintenance and distribution of live bio-agents made possible by preservation in culture collections.Discovering and maintaining bio-agents has wider ramifications in addition to improving our understanding of plant diseases. They support the creation of efficient disease management techniques, the safeguarding of agricultural output, and the conservation of plant genetic resources. These activities also allow researchers to track the formation of novel disease strains, analyse the evolution of pathogens, and pinpoint variables affecting pathogen adaptation. For the creation of agricultural systems that are robust and sustainable, this information is essential.

In general, finding and keeping bio-agents is essential for expanding plant pathology research, enhancing disease prevention methods, and guaranteeing the security of the world's food supply. Our ability to find, comprehend, and manage bio-agents will improve with continued work on developing novel detection methods, putting in place effective surveillance programmes, and perfecting preservation techniques, ultimately enhancing plant health, agricultural productivity, and sustainable development.

References

Agrios, G. N. (2005). *Plant pathology*.(5th eds.) Elsevier academic Press. *New York*.

APHIS, (2021). Animal and Plant Health Inspection Service. United States Department of Agriculture. Retrieved from https://www.aphis.usda.gov/.

Barrett, L. G. (2009). The impact of host resistance on the evolution and ecology of parasites. Evolutionary Applications, 2(3), 388-409.

Barton, J. H. (2005). Intellectual property rights and access to plant genetic resources for food and agriculture. Food Policy, 30(5-6), 405-426.

Benson, E. E. (2008). Cryopreservation of seeds and vegetative propagules. In Plant Conservation Biotechnology (pp. 137-162). Springer.

Boomsma, J. J., Jensen, A. B., Meyling, N. V., & Eilenberg, J. (2014). Evolutionary interaction networks of insect pathogenic fungi. *Annual Review of Entomology*, 59, 467-485.

Brown, S., Green, D. and Smith, J. 2020. Bio-Agents for the Control of Plant Diseases: A Review. *Plant Pathology Progress.* 26(4): 27-34.

CABI, (2020). Plant biosecurity manual. CABI Publishing.

Chakraborty, S. (2016). Emerging plant diseases: Recent trends, threats, and implications. In Plant Disease Epidemiology (Vol. 1, pp. 189-213). Springer.

Chiarolla, M. (2013). Intellectual property rights and plant variety protection: The interface between plants and biotechnology. In Patents for Chemicals, Pharmaceuticals, and Biotechnology (pp. 389-420). Oxford University Press.

Clark, M. F., & Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology,*34(3), 475-483.

Dasgupta, A. (2014). Methods in molecular biology (Vol. 1160): Immunological assays. Humana Press.

Doe, J., Jones, P. and Johnson, M. 2019. The Future of Bio-Agents in Plant Disease Management. *Plant Pathology Outlook.* 25(5): 35-42.

EPPO, (2021). European and Mediterranean Plant Protection Organization. Retrieved from https://www.eppo.int/.

FAO, (2021). International Plant Protection Convention (IPPC). Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/ippc/en/.

Fisher, M. C. (2012). Emerging fungal threats to animal, plant and ecosystem health. Nature, 484(7393), 186-194.

Fravel, D. R. (2005). Commercialization and implementation of biocontrol. *Annual Review of Phytopathology,*43, 337-359.

Garrett, K. A. (2011). *The lessons of emergence: Infectious diseases as models for ecological and evolutionary dynamics. Science,* 331(6020), 1564-1566.

Gaur, R. K., Khurana, S. P., Sharma, P., &Hohn, T. (Eds.). (2021). *Plant virus-host interaction: molecular approaches and viral evolution*. Academic Press..

Gnanamanickam, S., & Immanuel, J. (2006). Epiphytic bacteria, their ecology and functions. *Plant-associated bacteria*, 131-153.

Gould, J. F. (2016). Intellectual property management in plant pathology research. *Plant Disease,*100(2), 200-212.

Halewood, M. (2013). *Plant genetic resources for food and agriculture: Opportunities and challenges emerging from the science and information technology revolution. In The Role of Plant Genetic Resources in Food Security* (pp. 207-241). Springer.

Haque, Z., & Khan, M. R. (2021). *Handbook of Invasive Plant-Parasitic Nematodes: Novel Ingredients for Use in Pet, Aquaculture and Livestock Diets*. CABI.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., &Lorito, M. (2004). *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature reviews microbiology*, ***2***(1), 43-56.

Jones, J. D. G. (2016). Adaptive immunity to fungi. *Annual Review of Phytopathology,*54, 15-40.

Jones, P., Brown, S. and Green, D. 2022. The Role of Bio-Agents in Plant Disease Management. *Journal of Plant Protection.* 38(2): 11-18.

Jones, R. A. C. (2009). Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus Research,*141(2), 113-130.

Krattiger, A. F. (2007). *Intellectual property management in health and agricultural innovation: A handbook of best practices.* MIHR: Oxford.

Lacey, L. A., Frutos, R., Kaya, H. K., & Vail, P. (2001). Insect pathogens as biological control agents: do they have a future?. *Biological control*, 21(3), 230-248.

Lucas, J. A. (2009). *Plant pathology and plant pathogens*. John Wiley &Sons..

Mullis, K. B., &Faloona, F. A. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology,*155, 335-350.

Mundt, C. C. (2002). Use of multiline cultivars and cultivar mixtures for disease management. *Annual Review of Phytopathology,*40, 381-410.

Oh, S. H. (2018). Intellectual property rights in agriculture and plant biotechnology: Seeking a role in promoting innovation and food security. *Sustainability,*10(5), 1351.

Pal, S. (2018). Intellectual property rights (IPRs) and agriculture: A critical review. *Economic Affairs,*63(1), 181-195.

Pautasso, M. (2012). Emerging risks from plant pests and diseases under global change. *Journal of Pest Science,*85(2), 447-458.

Pautasso, M., Petter, F., Rortais, A., & Roy, A. S. (2015). Emerging risks to plant health: a European perspective. *CABI Reviews*, 1-16.

Pimentel, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics,* 52(3), 273-288.

Reed, B. M. (2008). *Plant cryopreservation: A practical guide.* Springer Science & Business Media.

Rumbos, C. I., &Athanassiou, C. G. (2017). Use of entomopathogenic fungi for the control of stored-product insects: can fungi protect durable commodities?. *Journal of pest science*, ***90***, 839-854.

Sharma, R. (2018). Intellectual property rights and agriculture: Emerging issues and challenges. *Agricultural Economics Research Review,*31(2), 291-304.

Smith, J., Doe, J. and Johnson, M. 2023. Uncovering and Preserving Bio-Agents in Plant Pathology. *Plant Pathology Journal.* 25(1): 1-10.

Stukenbrock, E. H. (2012). Evolution of fungal pathogens in agricultural ecosystems. *Annual Review of Phytopathology,*50, 461-483.

Subasinghe, R., & Shinn, A. P. (2023). Biosecurity: Current and Future Strategies. In *Climate Change on Diseases and Disorders of Finfish in Cage Culture* (pp. 430-461). GB: CABI.

Thrall, P. H. (2017). Rapid genetic adaptation to novel environments underpins the spread of a fungal pathogen. *Ecology Letters,*20(11), 1368-1376.

Van Lenteren, J. C., Bolckmans, K., Köhl, J., Ravensberg, W. J., &Urbaneja, A. (2018). Biological control using invertebrates and microorganisms: plenty of new opportunities. *BioControl*, 63, 39-59.

White, J., Black, E. and Blue, W. 2021. The Use of Bio-Agents in the Control of Plant Diseases. *Plant Disease Management Review.* 27(3): 19-26.

Wolfe, M. S. (1985). The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual Review of Phytopathology,*23, 251-273.

Ye, X. (2012). Recent advances in cryopreservation of shoot-derived germplasm of horticultural crops. *In Vitro Cellular & Developmental Biology - Plant,*48(5), 389-410.

11 Managing Plant Disease Vectors

V. M. Chaudhari[1] and D. C. Barot[1]

[1]Ph.D. Scholar, Department of Vegetable Science, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat.

Abstract

About half of the insect vectors are aphids, third and the leafhoppers. Mealy bugs and whiteflies transmit some viruses, and six are transmitted by thrips. The main aphid vectors are *Myzus persicae* (Sulzer), *Aphis gossypii* Glover and *Aphis craccivora* Koch. In addition, whitefly, *Bemisia tabaci* (Gennadius) and leafhoppers are also obligated for transmission of plant viruses. For inoculation of virus into a plant by sucking insects, the puncture is prepared by a number of forward and backward movements of the inner pair of stylets. Phytoplasmas are important insect-transfered pathogenic agents causing more than 700 diseases in plants. The single most promising order of insect phytoplasma vectors is the Hemiptera. Phytoplasmas are phloem-limited; therefore, only phloem-feeding insects can potentially acquire and transmit the pathogen. Most of the phytoplasma vectors are members of the Cicadellidae family. Pathogenic bacteria apparently cannot enter plants directly through unbroken cuticle but get in through insect or other wounds, stomata, hydathodes, lenticels and flower nectaries. The fact that the plant pathogenic bacteria are unable to penetrate plant tissue without a court of entry has led to the realization of significance of the role of insects in transmission. The insect contributes through feeding and oviposition wounds, as a mechanical carrier of the organism on its body and in some cases, by virtue of a mutualistic relationship between the organism and the insect which insures a continuing association among pathogen, insect and the host plant. More than 8,000 species are known to cause plant diseases. There are several insects associated with the tranfer of fungal diseases. Among the various types of plant

diseases transfered by insects, virus diseases are considered to be the most serious. Hence a multi-pronged strategy needs to be accepted to manage the vectors and virus diseases. Some of the important elements of such a strategy should involve selection of healthy seed, cultural practices, biological measures, resistant varieties and use of chemicals.

Keywords: Vectors, Viruses, phytoplasmas, Becterias, Fungus, Control

Introduction

A vector is an organism capable of transmitting pathogens from one host to another. The insects, besides directly damaging the crops, sometimes become responsible for the spread of microorganism that can cause disease in plants. All those insects which unload the disease causing organisms by feeding on the diseased plants, or by contact and transmit them to healthy plants are known as insect vectors of plant diseases.

Insect Vectors

Plant pathogens are transmitted either from contact, by contamination through soil or other biological agencies. Majority of the plant diseases are spread by insects and a few by other arthropods like mites and only a small percentage by mechanical means or contaminant of the soil.

Insects having both piercing and sucking mouthparts and biting and chewing mouthparts are associated with disease distribution. Most the insect vectors belong to the order Hemiptera (aphids, leafhoppers, whiteflies and mealy bugs), but a limited belong to Thysanoptera (thrips), Coleoptera (beetles), Orthoptera (grasshoppers) and Dermaptera (earwigs). Homopteran insects alone are known to transmit about 90 per cent of the plant diseases.

The salient features of homopterans (aphids and leafhoppers), which make them efficient vectors are as follows:

- » They make short but frequent probes with their mouthparts into host plants
- » As the population density reaches a critical level, winged migratory individuals are produced
- » In many species, winged females deposit a few progeny on each of the many plants
- » These insects do not induce wholesome destruction of cells during feeding and viruses require living cells for their subsistence and multiplication

A number of plant diseases induce by viruses, phytoplasmas, bacteria and fungi are transmitted by insects.

Viruses

A virus is a set of one or more nucleic acid molecules, normally encased in a protective coat or coats of protein or lipoprotein that is able to arrange its own replication only within suitable host cells. It is ultramicroscopic in size and can be seen only with the help of an electron microscope. It is spherical or rod-shaped, nucleoproteinaceous (chief constituents being ribonucleic acid 5-35% and proteins 65-95% by weight) in constitution and can live and multiply only in living cells. Viruses are obligated for many diseases in man (influenza, measles, mumps, polio, pox, etc.) and plants (mosaic, leaf curl, etc.). Plant virus diseases have become more prevailing and destructive in recent years. This is mainly because of better detection of the virus diseases, exchange of plant material from region to region facilitating spread of the virus to new areas, and distribution of many insect vectors in new regions in the world. There are over 850 described plant virus species. About half of the insect vectors are aphids, a third and the leafhoppers. Mealy bugs and whiteflies transmit some viruses, and six viruses are transmitted by thrips. The main aphid vectors are *Myzus persicae* (Sulzer), Aphis gossypii Glover and Aphis craccivora Koch. In addition, whitefly, *Bemisia tabaci* (Gennadius) and leafhoppers are also obligated for transmission of plant viruses. Whitefly mostly transfers mosaics and leaf curls in pulses, vegetables and other crops like cotton, tobacco and papaya. The leaf- and planthoppers transfer tungro, yellow-orange leaf, grassy stunt and ragged stunt in rice. Tomato spotted wilt is known to be conveyed by thrips. Mandibulate insects like grasshoppers, earwigs and chrysomelid beetles transfer turnip yellow mosaic (R. Paul, 2022). Several species of mites are also responsible for distribution of viruses of cereals and fruit crops (Table 14.1).

Table 14.1 List of important mite vectors of virus diseases of plants

S.No.	Mite vector	Virus	Host(s)
1.	*Abacarus hystrix* (Nalepa) (Acari: Eriophyidae)	Agropyron mosaic	Wheat, Switch grass
2.	*Aceria ficus* (Corte) (Acari: Eriophyidae)	Fig mosaic	Fig
3.	*Aceria tulipae* (Keifer) (Acari: Eriophyidae)	Wheat streak mosaic	Wheat, Oats, Barley, Maize
4.	*Aculus fockeui* (Nalepa & Trouessart) (Acari: Eriophyidae)	Prunus necrotic ring spot	Plum, Peach, Cherry
5.	*Eriophyes inaequalis* Wilson & Oldfield (Acari: Eriophyidae)	Cherry leaf mottle	Sweet cherry

6.	*Eriophyyes insidiosus* Keifer & Wilson (Acari: Eriophyidae)	Peach mosaic	Peach, Nectarine

Source: Gillot (2005)

Types of Viruses:

On the basis of the method of transmission and persistence in the vector, viruses may be classified into three categories:

(i) Non-Persistent Viruses

These are those viruses which are believed to be spreaded as contaminants of the mouthparts. Such viruses are also called stylet-born viruses and the type of distribution is mechanical. The vector is able to acquire the virus from a disease source and spread to a healthy plant by feeding for a few seconds. These viruses do not persist longer within the insect vectors which can spread them soon after feeding on infected plant but the ability to transfer fresh infection soon disappears after the insect feeds on healthy or immune plants. The efficiency of distribution of non-persistent virus is greatly affected by modifying the time of feeding and by starving the vectors before and after feeding. Aphids are the vectors of majority of such viruses which are carried only in their stylets.

The following are the main features of the non-persistent viruses

- Vectors are optimally infective when they have fed for approximately 30 seconds on the infected plant
- Transmission is improved if vector is starved for a period before an infection feed
- If the vector is starved after an acquisition, it begins to lose ability to transfer within 2 minutes
- After acquisition feeding, infectivity is rapidly lost when the vectors feed on healthy plants.

(ii) Semi-Persistent Viruses

These viruses are carried in the anterior regions of the gut of a vector, where they may multiply to a certain extent. Vectors do not normally remain infective after a molt, presumably because the viruses are lost when the foregut intima is shed. Several of the leafhopper transferred viruses fall under this category.

(iii) Persistent Viruses

Persistent viruses are those that persist longer within the infective agent, *i.e.*, vector. These viruses, when superficial by a vector, pass through the midgut wall to the salivary glands from where they can infect new hosts. In case of these viruses, the insect has to feed on the source of virus for comparatively longer periods. The insect, after such accession of virus, becomes infective only after a certain period, ranging from several hours to 10-20 days, which is called the incubation period or latent period. Such viruses may multiply within tissues of a vector, which retains the ability to transfer the virus for several days and in some instances the rest of its life. Therefore, the vector need not feed on the virus source again and again to reserve its infective capacity. Thus, the vector insect feeds on the diseased plant (acquisition feed), needs some time after acquisition feed to transmit the virus (latent or incubation period), feeds on healthy plant (inoculation feed) and in the process transfers the virus acquired earlier. This type viruses are also called circulative or circulative-propagative viruses and the type of transmission as non-mechanical. Many of the leafhopper transmitted viruses belong to this category.

Mechanism of Transmission

For inoculation of virus into a plant by sucking insects, the puncture is prepared by a number of forward and backward movements of the inner pair of stylets. During the forward movements, the fluid flows into them, during the backward movements, saliva is ejected. Generally, an insect injects by feeding on any part of the plant, but in some cases the virus is only found in the phloem and has to be injected into the phloem, the movement of which is probably controlled by the pH gradient between the mesophyll and the phloem. Some viruses are concerted in the epidermal cells and others in the mesophyll or xylem. The orthopteran insects like grasshoppers and beetles regurgitate during feeding. The regurgitated fluid containing the virus is brought into contact with the healthy plant, thus transmitting the virus.

Virus-Vector Relationship

Irrespective of the type of transmission, virus-vector relationship is highly specific. Generally, one type of virus disease is transferred only by insects belonging to one particular group, *i.e.*, mosaics by aphids and leaf curls by whiteflies. In case of leafhoppers, among 110 species known to be vectors, about 100 species spread only one virus. Similarly, there are viruses which are transferred by a particular species of an insect and not by others of the same genus. For instance, cabbage ring spot is transfered only by *M. persicae* and not by *M. ornatus*. A vector can also acquire and transfer more than one virus to the respective hosts. For example, the aphid,

Pentalonia nigronervosa Coquerel, transfer banana bunchy top and cardamom mosaic. Similarly, the whitefly, *Bemisia tabaci* (Gennadius) transmits okra yellow vein mosaic, dolichos yellow mosaic, tomato leaf curl, papaya leaf curl, *etc*. Onion yellow dwarf is known to be transmitted by 60 insect vectors. For distribution of viruses, activity of insect vectors is more important rather than their number. In case of aphids, it is the activity and number of migrant insects that is important in the efficiency of virus transmission rather than the number of apterous individuals which are, of course, important in respect of their direct injury to the crop (Hemmati *et al*., 2023).

Phytoplasmas

Phytoplasmas (originally called mycoplasma-like organisms) are non-culturable degenerate gram-positive prokaryotes closely related to mycoplasmas and spiroplasmas. They are without a visible cell wall, whose place is taken by a thin elastic cytoplasmic membrane which cannot resist osmotic pressure. Phytoplasmas are pleomorphic and may be spherical or oval, varying from 80 to 800μ in diameter. Phytoplasmas are important insect-transfered pathogenic agents causing more than 700 diseases in plants. The single most promising order of insect phytoplasma vectors is the Hemiptera (Weintraub and Beanland, 2006). This group collectively possesses some characteristics that make its members efficient vectors of phytoplasmas.

- » They are hemimetabolous, thus nymphs and adults feed similarly and are in the same physical location-often both immatures and adults can transmit phytoplasmas
- » They feed specifically and selectively on certain plant tissues, which makes them efficient vectors of pathogens residing in these tissues
- » Their feeding is nondestructive, promoting successful inoculation of the plant vascular system without damaging the conductive tissues and eliciting defensive responses
- » They have a reproductive and persistent relationship with phytoplasmas. They have obligate synergetic prokaryotes that are passed to the offspring by transovarial transmission, the same mechanisms that allow the transovarial transmission of phytoplasmas.

Mechanism of Transmission

Phytoplasmas are phloem-limited; therefore, only phloem-feeding insects can potentially acquire and transmit the pathogen. Most of the phytoplasma vectors are members of the Cicadellidae family. Phloem-feeding insects acquire phytoplasmas passively during feeding in the phloem of infected plants. The feeding duration

necessary to acquire a adequate titer of phytoplasma (acquisition access period), may range from a few minutes to several hours, the longer the period, the greater the chance of acquisition. The time that elapses from initial acquisition to the ability to transfer the phytoplasmas (latent period or incubation period) is temperature dependent and ranges from a few to 80 days. During the latent period, phytoplasmas move through and reproduce in the vector's body. They can pass intracellularly through the epithelial cells of the midgut and replicate within a vesicle or they can pass between two midgut cells and through the basement membrane to enter the hemocoel. Phytoplasmas circulate in the haemolymph, where they may infect other tissues such as the Malpighian tubules, fat bodies and brain or reproductive organs. The replication in these tissues, albeit not essential for transmission, may be indicative of a longer coevolutionary relationship between host and pathogen. To be transferred to plants, phytoplasmas must penetrate specific cells of the salivary glands and high levels must accumulate in posterior acinar cells of the salivary gland before they can be transferred.

Vector-Phytoplasma Relationship

The relation between insects and phytoplasmas is complex and variable. The complex sequence of events required for an insect to acquire and subsequently transfer phytoplasmas to plants suggests a high degree of specificity of phytoplasmas to insects. However, numerous phytoplasmas are transfered by several different insect species. In addition, a single vector species may spread two or more phytoplasmas, and an individual vector can be infected with dual or multiple phytoplasma strains. Vector-host plant relations also play an important role in determining the spread of phytoplasmas. Polyphagous vectors have the potential to inoculate a wider range of plant species, depending on the protest to infection of each host plant. It has been found that leafhoppers are not able to acquire equally phytoplasmas from different infected plant species. Chrysanthemum yellows (CY) phytoplasma is successfully transfered by three leafhoppers, *viz.*, *Euscelidius variegatus, Macrosteles quadripunctulatus* and *Euscelis incisus*. All three species acquire from and transfer to CY-infected chrysanthemum and uninfected chrysanthemum, respectively. However, only *M. quadripunctulatus* and *E. variegatus* acquire CY after feeding on CY-infected periwinkle and subsequently spread CY to uninfected plants. None of the leafhoppers acquire the phytoplasma from CY-infected celery, a dead-end host. Dead-end hosts are plants that can be inoculated and subsequently become infected with phytoplasma, but from which insects cannot acquire phytoplasma.

Bacteria

Bacteria are microscopic single celled organisms increasing by fission; they have a

cell membrane, a rigid cell wall and often one or more flagella. Of the total about 1800 known bacterial species, most are saprophytes living on dead plant or animal tissues or organic wastes. There are about 200 species of bacteria which are parasitic on plants and many of them consisting of numerous pathovars.

Bacterial diseases fall into three categories

(i) Wilting, due to invasion of the vascular system or water-conducting vessels, *e.g.*, cucumber wilt.

(ii) Necrotic blights, rots and leaf spots, where the parenchyma is killed, *e.g.*, fire blight.

(iii) Hyperplasia or over growth, *e.g.*, crown gall.

Pathogenic bacteria apparently cannot enter plants directly through unbroken cuticle but get in through insect or other wounds, stomata, hydathodes, lenticels and flower nectaries. The fact that the plant pathogenic bacteria are unable to penetrate plant tissue without a court of entry has led to the realization of significance of the role of insects in transmission. The insect contributes through feeding and oviposition wounds, as a mechanical carrier of the organism on its body and in some cases, by virtue of a mutualistic relationship between the organism and the insect which insures a continuing association among pathogen, insect and the host plant. A number of plant diseases caused by bacteria are known to be transmitted by insects . Fire blight of apple and pear, caused by *Erwinia amylovora* is carried by aphids, leafhoppers, *etc*. Potato blackleg, caused by *Erwinia carotowora*, is transfered by seed corn maggot, *Hylemyia cilicrura* (Rondani) (Chandi *et al*., 2018). Bacterial wilt of cucurbits, caused by *Erwinia tracheiphila* is transfered by cucumber beetle, *Diabrotica duodecimpunctata* (Olivier). Bacterial wilt of maize, caused by *Xanthomonas steward* is transfered by the flea beetle, *Chaetocnema pulicaria* (Meisheimer). Black rot of crucifers, caused by *Xanthomonas campestris* is transmitted by several insects and slugs.

Fungi

Fungi are organisms having no chlorophyll, reproducing by sexual and asexual spores, not by fission like bacteria and typically having a mycelium or mass of interwoven threads (hyphae) containing well marked nuclei. There are about 4300 valid genera of fungi and about 70,000 species living as parasites or saprophytes on other organisms or their residua. More than 8,000 species are known to cause plant diseases. There are several insects associated with the tranfer of fungal diseases. Many flies mechanically transfer the ergot of cereals caused by *Claviceps purpurea*. The ergot disease of bajra, caused by *Sphacelia microcephala*, is mechanically conducted by insects that visit the flowers attracted by the sugary secretion found on the fungus infected earheads. The cotton wilt, caused by *Fusarium vasinfectum*, is transfered through the faecel

pellets of many grasshoppers like *Melanoplus differentialis* (Thomas), after they have fed upon infected plants. The common sooty mould fungus (*Capnodium spp*.) grows on the honeydew excreted by several homopteran insects like aphids, leafhoppers, mealy bugs, whiteflies, *etc*.

Control of Vectors and Diseases

Among the various types of plant diseases transfered by insects, virus diseases are considered to be the most serious. Hence a multi-pronged strategy needs to be accepted to manage the vectors and virus diseases. Some of the important elements of such a strategy should involve selection of healthy seed, cultural practices, biological measures, resistant varieties and use of chemicals.

(i) Healthy Seed

Management of virus diseases starts with obtaining healthy seed, cuttings or plants. Care should be taken to acquire only certified seed, *i.e*. seed obtained from the plants which have been inspected during growing season and found free of certain diseases. Virus-free foundation stock can be built up by heat treatment, *i.e*., growing plants at high temperatures for weeks or even months. The production of virus-free stocks can also be achieved by taking benefit of the fact that some plants grow and elongate faster than the virus can occupy the new tissue. Therefore, the virus can be removed by using meristem or tip cultured plants. Virus free stock is tested by indexing (growing a part of the cutting or plant in a pot or greenhouse and record its condition with respect to disease symptoms), bioassays and/or serological assays.

(ii) Cultural Control

Several cultural practices have proved to be helpful in reducing the incidence of vectors and vector-borne diseases. Intercropping with a barrier crop has provided encouraging results to reduce the incidence of various diseases. For example, the incidence of yellow vein mosaic of okra is decreased by intercropping with soybean. Similarly, intercropping of tomato with coriander and lobiabean decrease the incidence of tomato leaf curl virus in tomato. Plant spacing such as close spacing lower the incidence of French bean crinkle stunt disease. Manipulation in planting dates is another way of decreasing the disease incidence. Rogueing also helps the deletion of the source of disease causing organism. Removal of weeds and alternate hosts of viruses and vectors helps to lower the incidence of diseases.

(iii) Resistant Varieties

Growing resistant/tolerant varieties is another effective way of managing vectors and vector- transmitted diseases. A number of genotypes have been identified under

various All India Coordinated Research Projects, supported by the various State Agricultural Universities and Indian Council of Agricultural Research, which have resistance against virus borne diseases in pulses, tomato, cotton, *etc*.

(iv) Biopesticides

The use of biopesticides such as parasitoids and predators, microbials and plant extracts is an eco-friendly approach to control the vectors and vector born diseases. A fungus, *Paecilomyces farinosus* has been found parasitic on *Bemisia tabaci* (Gennadius). Various neem-based formulations have provided effective control of *B. tabaci* on cotton. Aqueous extracts of leaves of *Clerodendron frageans* and *Aerva anguinolenta* and roots of *Boerhavia diffusa*, sprayed at 4 per cent concentrations at 3-4 days starting from germination, was found to decrease yellow mosaic incidence in blackgram and mungbean.

(v) Chemical Control

The control of insect vectors by application of insecticides appears to be a difficult task as few survivors would be able to transmit the disease. Still insecticidal control of insect vectors is the most practicable method of control of plant viruses. The timely application of insecticides restricts the spread of the disease by decreasing the vector population. Several systemic and non-systemic insecticides have been reported to control the insect vectors. The prominent insecticides reported to be effective are sprays of oxydemeton methyl, malathion, dimethoate, carbaryl, dichlorvos, fenitrothion, phosphamidon, monocrotophos, triazophos and ethion in doses ranging from 300 ml to 1-5 litres per ha. The soil application of carbofuran and phorate granules @ 10-12 kg per ha has also proved useful.

Conclusion

Plant disease which spread by vector are controlled by controling the vector throgh it spread. Management of virus diseases starts with obtaining healthy seed, cuttings or plants. Care should be taken to acquire only certified seed. Several cultural practices have proved to be helpful in reducing the incidence of vectors and vector-borne diseases. Intercropping with a barrier crop has provided encouraging results to reduce the incidence of various diseases. Growing resistant/tolerant varieties is another effective way of managing vectors and vector- transmitted diseases. The use of biopesticides such as parasitoids and predators, microbials and plant extracts is an eco-friendly approach to control the vectors and vector born diseases. The control of insect vectors by application of insecticides appears to be a difficult task as few survivors would be able to transmit the disease. Still insecticidal control of insect vectors is the most practicable method of control of plant viruses.

References

Chandi, R. S.; Kataria, S. K. and Kaur, J. (2018). Arthropods as vector of plant pathogens viz-a-viz their management. *Int. J. Curr. Microbiol. App. Sci.,* **7**(8): 4006-4023.

Gillott, C. (2005). Entomology. **pp**:276.

Hemmati, C.; Nikooei, M.; Tiwari, A. K. and Al-Sadi, A. M. (2023). Management of insect vectors associated with phytoplasma diseases. *Phyto. Diseas. Asian Countr.*, **3**: 125-136.

Paul, R. (2022). Insect vectors of plant diseases and its control. *Agri. India*, Chap. 31 **pp:** 1-10.

Weintraub, P. G. and Beanland, L. (2006). Insect vectors of phytoplasmas. *Annu. Rev. Entomol.,* **51**: 91-111.

12 Mushroom Production Technology and Value-Added Products

Padsala J. J.[1*], Joshi R. L.[1], Shekhada H. A.[2] and Bhaliya C. M.[3]

[1]Ph.D. Scholar, Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat - 396450
[2] Ph.D. Scholar, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat - 362001
[3] Assistant Professor, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat - 362001

Abstract

Mushrooms have numerous health and nutritional advantages and can help in several kinds of malnutrition and undernutrition issues. Despite this, due to their high perishability, mushroom cultivation and consumption are not growing quickly. In most horticultural products, post-harvest losses are very excessive, and they may be highest for mushrooms. Even after being harvested, mushrooms continue to develop, respire, mature and senesce, which leads to weight loss, veil opening, browning, wilting, diseases, and ultimately spoilage. Therefore, effective production technology use is also essential in production units. Thus, it is vital to process mushrooms into value-added products which will not only provide the protein and micronutrient necessity of the public but at the same time will solve the problem of short shelf-life and post-harvest losses of mushrooms. Here are a few technologies for turning mushrooms into high-value products with long shelf lives. I have discussed some significant value-added goods in this chapter as well as the stages involved in the mushroom production method.

Keywords: Mushroom, value addition, soup powder, pickle, biscuit, cosmetic, preservation

Introduction

Mushrooms are incredibly nutrient-dense and adaptable living things that have grown in popularity in a variety of culinary and therapeutic applications (Pandey *et al.*, 2022 and Sahoo *et al.*, 2022). Due to their distinctive flavours, high nutritional value and potential health benefits, mushrooms have been progressively becoming more and more popular. The technology used in mushroom production describes the procedures and methods used in industrial-scale mushroom cultivation (Fig. 1). The various facets of mushroom production technology, such as substrate preparation, spawn production, growing methods and environmental control, will be briefly discussed in this chapter.

Production Technology

Substrate Preparation

The preparation of the substrate is an essential phase in the culture of mushrooms since it supplies the nutrients and support needed for mushroom growth. The species of mushroom being grown will determine the substrate to use. Agricultural waste products including straw, cake, sawdust, wood chips and several kinds of compost are examples of common substrates (Gowda and Manvi, 2019 and Mohd Hanafi *et al.*, 2018).

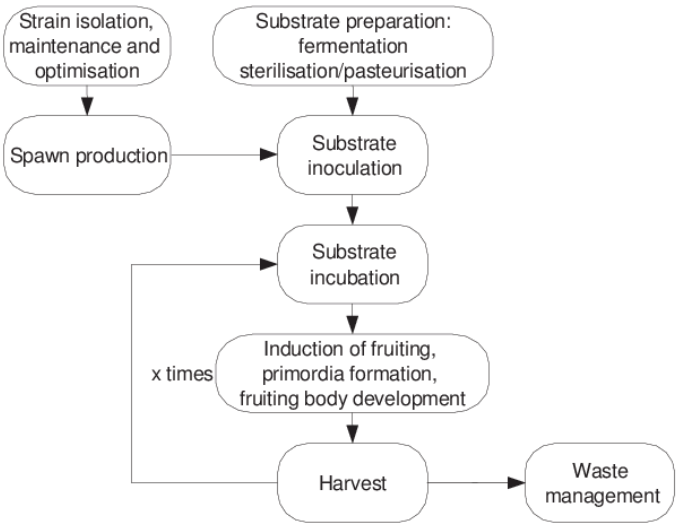
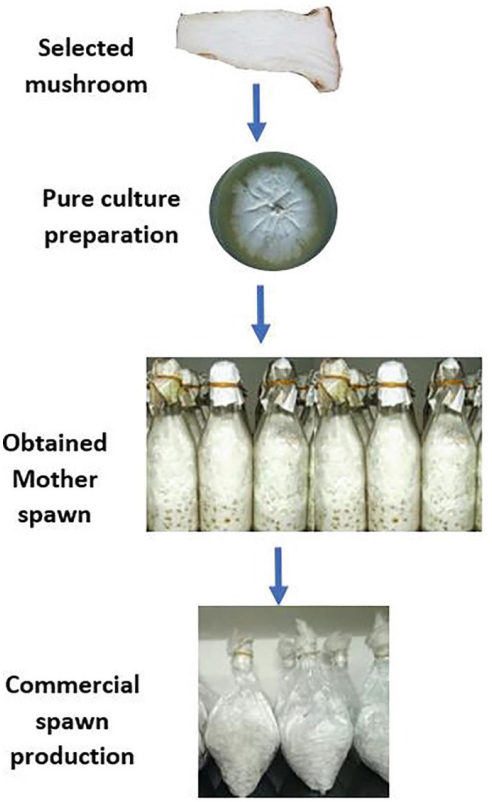


Fig. 1: Flow chart for the production technology of mushroom

The substrate preparation process comprises numerous steps, including chopping, grinding, sterilization and supplementation (Cunha Zied *et al.*, 2020). The substrate should be finely chopped or ground to provide a suitable texture for the mycelium to colonize. Sterilization is essential to eliminate any competing microorganisms that could hinder mushroom growth. Supplementation with nitrogen, minerals and other nutrients may be necessary to optimize the substrate's nutritional content.

Spawn Production



Spawn serves as the inoculum for mushroom cultivation. It consists of a substrate colonized by mushroom mycelium and is used to introduce the mycelium into the cultivation substrate. Spawns can be produced through various methods, including laboratory-based techniques and outdoor methods.

In laboratory-based spawn production, mycelium is grown on a sterile substrate, such as agar or grain, in a controlled environment. The mycelium is then transferred to a larger volume of the sterilized substrate to produce a larger quantity of spawn.

Mycelium is grown on a substrate, such as grains or sawdust, in outdoor beds or containers as part of the production of spawn outside. The substrate is given time to become colonized by the mycelium and the spawn that results is used to grow mushrooms.

Cultivation Techniques

In the production of mushrooms, various cultivation methods are employed, including the following:

1. Bed Cultivation

Bed cultivation entails setting up a sizable substrate and spawn-filled bed. To make a stable, even bed, the substrate-spawn mixture is carefully stacked and compressed. A layer of casing material, like peat moss or vermiculite, is then placed on top of the bed, helping to control moisture levels and creating an ideal environment for fruiting.

2. Container Cultivation

For mushroom species that demand a more regulated environment, container cultivation is appropriate. You can use plastic bags or containers that have been filled with a substrate-spawn mixture. To produce a sterile environment, the containers are sterilized, filled with the mixture and sealed. After that, the containers are incubated in a regulated setting until the mycelium has completely colonized the substrate. The containers are opened to allow fruiting after colonization is finished.

3. Log Cultivation

Growing specialty mushrooms like shiitake or oyster mushrooms on logs is a frequent method. This method involves injecting spawn into hardwood tree logs. The logs are subsequently set in a shady area or a log-growing environment that has been specifically created. The logs become colonized by the mycelium throughout time and fruiting bodies develop.

Bed Cultivation

Container Cultivation

Log Cultivation

Environmental Control

To successfully grow mushrooms, an ideal atmosphere must be established. Humidity, temperature, light, and airflow are just a few variables that can have a big impact on how mushrooms grow and establish (Cliffe and O'beirne, 2010; Bellettini *et al*., 2020).

Temperature: For optimum growth, many types of mushrooms possess distinct temperature necessities. To keep an appropriate temperature spectrum, temperature regulation equipment like heaters or coolers may be utilized (Mahajan *et al*., 2008).

Humidity: High humidity levels must be present for the optimal development of mushrooms (Mahajan *et al*., 2008 and Bellettini *et al*., 2020). This may be done by misting often, using humidifiers, or by using a humidification system to create a precise atmosphere.

Light: The majority of mushrooms favour low light conditions or indirect light. However, some species want particular lighting conditions in order to ripen. The majority of mushrooms like dim or indirect lighting (Bellettini *et al*., 2020). Some species, however, need particular lighting conditions in order to ripen. The right lighting setups, like fluorescent or LED lights, can be implemented to suit the growing mushrooms' lighting needs.

Air Movement: In order to supply fresh oxygen and eliminate carbon dioxide, there must be an adequate air exchange. In order to ensure optimum circulation of air inside of the growth space, ventilation mechanisms or fans should be placed (Bellettini *et al*., 2020).

Role of Value Addition in Mushroom

In addition to being a common culinary item, mushrooms also contain a number of beneficial substances which may be utilized to create products with additional value. The demand for individual portion packs and convenience formats in every day diet due to women empowerment and lack of time. Hence, the demand for appropriate products of mushroom increased (BordGlas, 2002). In the food, pharmaceutical, nutraceutical, and cosmetic sectors, these items have several kinds of uses. Mushrooms are unable to endure storage for a period of time exceeding 24 hours in tropical ambient temperatures due to their high moisture content and fragile texture. Weight loss, veil opening, browning, liquefaction, and microbiological decomposition frequently render the product completely unusable.

Effective processing methods will increase both the compensation for growers and processors while reducing postharvest losses. The value-added products made from mushrooms will be thoroughly examined in the following section, along with their manufacturing procedures and possible advantages. The production processes

for a few selected value-added items will also be illustrated with flowcharts.

Value-Added Products from Mushrooms

1. Mushroom soup powder

A flexible value-added product, mushroom powder can be employed as a functional component in a range of foods, supplements and formulations. The nutritious and bioactive components found in mushrooms are retained in mushroom powder, which also provides convenient storage and prolonged shelf life. People who are watching their diet frequently eat soups as a main entrée. A high-quality, ready-to-use powder for mushroom soup made from button and oyster mushrooms that have been dried in a dehumidifying air cabinet dryer. The following steps, which are described below, are commonly used in the manufacturing of mushroom powder (Fig. 2). (Wakchaure, 2011)

Table 1: Ingredients for mushroom soup powder

Ingredients	Parts (%)
Mushroom powder	16
Corn flour	5
Milk powder	50
Refined oil	4
Salt	10
Cumin powder	2
Black pepper	2
Sugar	10
Ajinomoto	2

Raw Material Preparation: Cleaning and selection of fresh mushrooms are done to prepare the raw materials. For appropriate particle size, materials should be chopped, sliced, or crushed.

Drying: After being passed via a 0.5 mm sieve, dried button mushroom slices or whole oyster mushrooms were ground to a fine powder in a pulveriser. By combining this powder with milk powder, maize flour and other substances, mushroom soup powder can be made (Table 1). The following is a detailed flow chart (Fig. 2) of the steps involved in making mushroom soup powder. To make high-quality mushroom soup with a distinct flavour and aroma, combine this with an equal amount of water. Singh (1996) used vacuum-concentrated whey, a byproduct of the dairy industry, to create a powder for mushroom soup that is ready to be reconstituted.

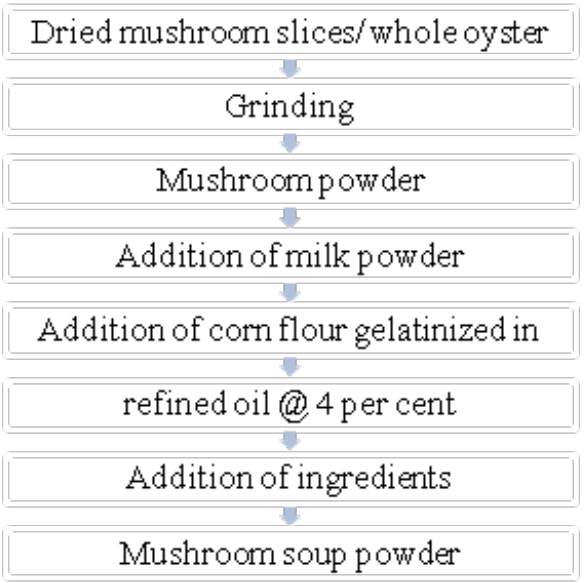


Fig. 2: Flow chart for preparation of mushroom soup powder

2. Mushroom biscuit

At NRCM, Solan, button and oyster mushroom powder, as well as ingredients maida, sugar, oil, baking powder, ammonium bicarbonate, salt, vanilla, milk powder, and glucose, were used to make very tasty and crunchy mushroom biscuits (Table 2) (Wakchaure, 2011). Oil-based fat is added to biscuits to improve their softness and reduce their hardness, which reduces the force needed to break and crush the biscuits.

Table 2: Ingredients for mushroom biscuits

Ingredients	Parts (%)
Maida	100
Sugar	30
Fat	45
Baking powder	0.6
Ammonium bicarbonate	0.3
Salt	0.6
Vanilla essence	0.02
Milk powder	1.5
Glucose/Fructose	1.5
Water	12 to 22

Sugar is the primary sweetening agent, and the Maillard reaction and polymerization it causes result in baked biscuits with heightened colour. Sugars have an impact on how flour proteins become denaturized by heat. The aerating agent ammonium carbonate provides the benefit of leaving no residue and evaporating more gas per unit weight than any other aerating agent, but because phosphate and sodium ions are absent, it gives the biscuits a strange flavour.

Table 2 provides a list of the different ingredients needed to prepare mushroom biscuits. The preparation flow chart for mushroom biscuits is shown in Figure 3 below. For 3 to 5 minutes, all the ingredients must be blended in a mixer. The dough is baked for 10-12 minutes at 210°C in a laboratory baking oven after being stored at 30°C for 90 minutes. It is then spread to a thickness of 2-4 mm on a prepared platform and cut into the necessary shapes (circular or rectangular).

3. Mushroom nuggets

In North India, 'Nuggets' are typically made from 'pulse' powder, such as black gram powder, soybean powder, urad dhal powder, etc., which is utilized to produce vegetable

curry. Since they are made from pulse powder, the nuggets give the meal flavour as well as nutrients. To make mushroom nuggets, 'Urad' dhal powder and mushroom powder (dried and coarsely crushed mushrooms) are combined, and then the necessary amount of water is added to form a paste. The prepared paste is mixed with ingredients and spices, and then spherical balls with a diameter of 2-4 cm are formed from the dough. To create the mushroom nuggets, the prepared balls are spread out over a steel pan and dried using the sun-drying method. The ingredients used in the preparation of nuggets by NRCM, Solan are listed in Table 3 (Wakchaure, 2011).

Table 3: Ingredients for mushroom nuggets

Ingredients	Parts (%)
Mushroom powder	10
Urad dhal powder	80
Salt	2
Red chilly powder	1
Sodium bicarbonate	0.01
Water	7

These nuggets can be enjoyed in two different ways: they can be deep-fried right away and used as snacks, or they can be added to vegetable curries either alone or with other suitable vegetables. The nugget preparation flowchart is shown in Figure 3.

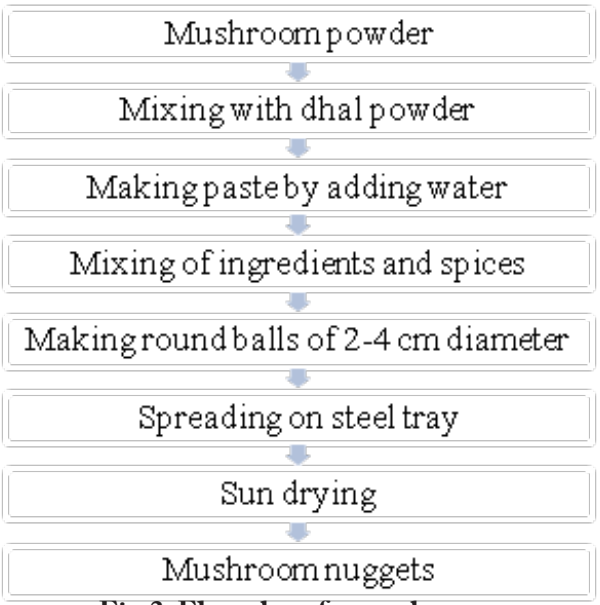


Fig.3: Flow chart for mushroom nuggets

4. Mushroom ketch-up

A common and well-liked product, ketchup is often served as a snack food accompaniment because of its familiar flavour and texture. It is made by concentrating the juice or pulp of the fruits or vegetables, removing the seeds and skin pieces because they detract from the ketchup's aesthetic appeal. It is extremely viscous and does not flow easily. Additionally, they have a higher sugar to acid ratio.

Freshly harvested button mushrooms are cleaned, thinly sliced, and cooked for 20 minutes in 50 per cent of water. Use of a mixer grinder is required to make mushroom paste. The paste is combined with arrarote (0.2%), acetic acid (1.5%) and additional ingredients (listed below) before being cooked to a TSS of 35^0Brix. The ketchup is then put into the clean bottles or jars. Table 4 contains a list of all the ingredients needed to make mushroom ketchup.

Table 4: Ingredients for mushroom ketch-up

Ingredients	Parts (%)
Salt	10
Sugar	25
Acetic acid	1.5
Sodium benzoate	0.065
Onion	10
Garlic	0.5
Ginger	3
Cumin	1
Black pepper	0.1
Red chili powder	1
Azinomoto	0.2
Arrarote	0.2

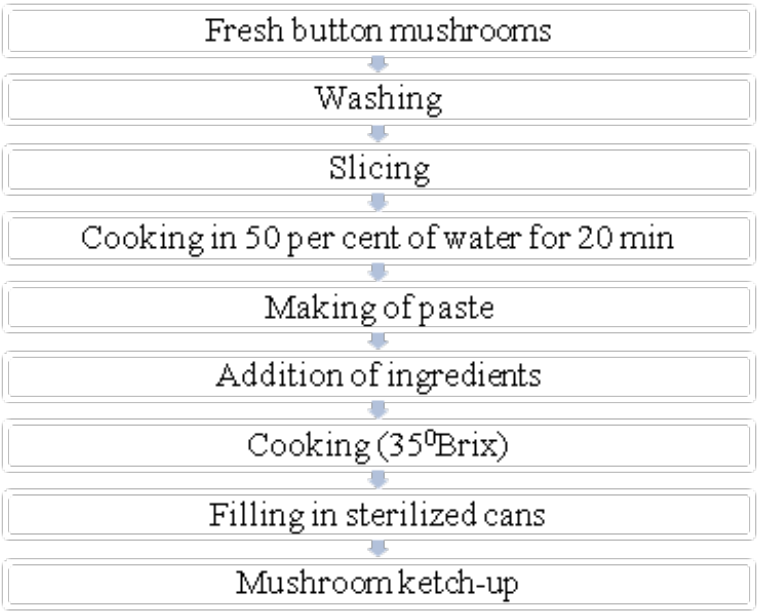


Fig. 4: Flow chart for mushroom ketchup

5. Mushroom candy

The process for making candy is essentially the same as that used in the case of mushroom maintenance described elsewhere, with the difference that the produce is impregnated with a higher concentration of sugar. The total sugar content of the impregnated produce is kept at about 75 per cent to prevent fermentation and the impregnated produce is then taken out and dried. (Wakchaure, 2011)

After being harvested, fresh mushrooms are washed and cut in half longitudinally. Slices are blanched in a 0.05 per cent KMS solution for 5 minutes. These are treated with sugar after draining for 30 minutes. 1.5 kg of sugar is added for every kg of blanched mushrooms during the sugar treatment. The sugar must first be separated

into three equal portions. On the first day, blanched mushrooms are kept for 24 hours while being covered with one part sugar.

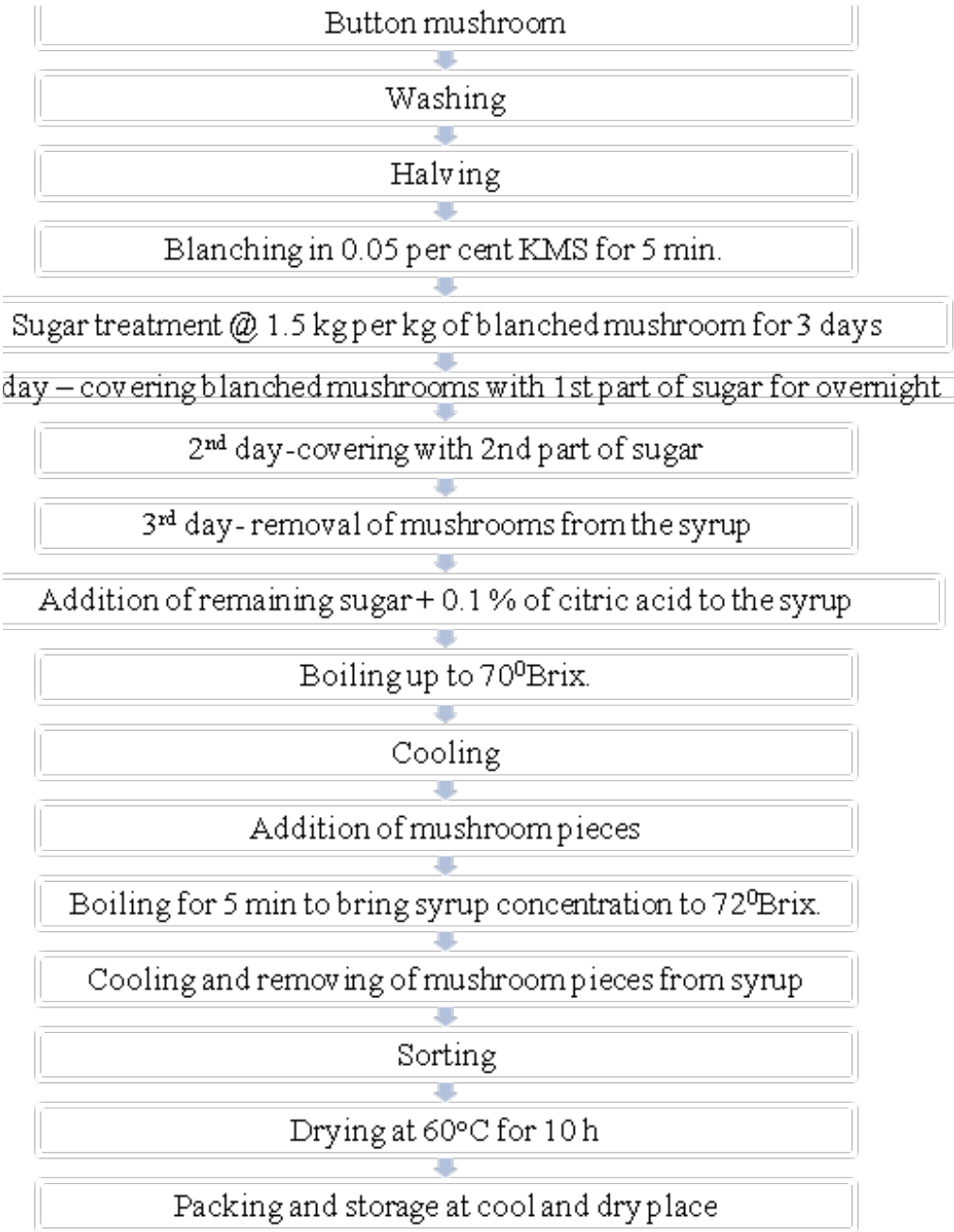


Fig. 5: Flow chart for mushroom candy

The same mushrooms are covered with a second layer of sugar the following day, left overnight, and then taken out of the sugar syrup the following day. To get the sugar syrup's concentration to 70°Brix, it is boiled with the third part of the sugar and 0.1% citric acid. This syrup is combined with mushrooms and the mixture is subsequently boiled for five minutes to increase the concentration to 72°Brix. The mushrooms are taken out of the syrup after cooling and allowed to drain for 30 minutes. The dried mushrooms are spread out on the sorting tables to be separated, removing any damaged or extra pieces.

The final step involves drying the mushroom pieces for roughly 10 hours at 60°C in a cabinet dryer. All mushrooms are removed as soon as they start to turn crispy, then sealed in polypropylene bags. The chewable mushroom candies (Fig. 5) have excellent acceptability and taste and can be kept for up to 8 months. Figure 5 shows the process flow for making candy from button mushrooms.

6. Mushroom Pickle

Mushroom canning may not be possible in our circumstances, particularly in rural areas. Making pickles is a common alternative in the area. Some mushrooms, such as milky mushrooms, are actually more popular as pickles than as fresh products. Pickling is a simple, at-home procedure for preserving mushrooms into a highly marketable value-added product. One kilogramme of mushrooms is washed in a 0.03 to 0.05 percent KMS solution before being blanched for five minutes at 85 °C to make mushroom pickle. (Markam, (2014)

The mushrooms are drained after being rinsed in cold water two to three times. Depending on their size, mushrooms are cut into halves or quarters. The next step is salt curing, which involves adding 20 g of sodium chloride per kilogram of mushrooms and enabling them to sit for an overnight period. The following day, the excess water that oozes out of the mushroom is removed. After allowing them to dry for a couple of hours, mushrooms are mixed with seasonings, salt, and preservatives to achieve the desired flavour and quality of mushroom pickle. We fill plastic or glass jars with the mixture and again top-fill with heated mustard oil. We then screw on the lids, seal the jars, and store the pickles in a cool, shaded location overnight to allow the spices to absorb the oil and develop their flavour.

Ingredients	For 1kg
Turmeric powder	20g
Black mustard seed powder	35g
Red chilli powder	10g
Cumin seed powder	1.5 g
Carom seed	10 g
Nigella seed (kalonji)	10 g
Fennel seed powder	1.5 g
Salt	90 g
Mustard oil	200 ml

Additionally, up to 100 ml of acetic acid and the permitted amounts of sodium benzoate may be used as preservatives. Within airtight containers, this pickle can be kept for up to a year.

7. Mushroom Papad

Papad is an Indian snack food that usually consists of a thin, crisp batter made of peeled black gramme flour (urd flour), lentils, chickpeas, rice, tapioca, or potatoes and fried or cooked over dry heat. Mushrooms can be used to boost the protein content of papads in a form of paste or dried powder in the batter made from the previously mentioned other sources. As a result, papad may become a nutritious food with a high protein content. As per Markam (2014), process can be summed up as follows:

- Wash harvested mushrooms in water (containing 300 ppm KMS).
- Blanch mushrooms in hot water at 85°C for 5 min and then dip immediately in cold water.
- Cut mushrooms into halves or quarters and make a paste in commercial/ kitchen mixer grinder.
- Boil potatoes in a pressure cooker or vessel, peel and crush/grate boiled potatoes to a paste consistency
- Blend potato paste and mushroom paste in 50:50 ratio and add spices (Salt, chili powder/ black pepper, cumin etc as per taste).

» Add KMS powder to the blend @ 300 ppm.

» Spread the papad batter on a polyethylene sheet in drying trays of inappropriate round size (10 cm diameter) with uniform thickness (1-2 mm).

» Keep the trays in the sun for sun drying or in a tray drier at 50°C for 5-6 hr to reduce the moisture content to 5%.

» Peel off the dried papads from the polyethylene film and pack them in sets of 10-20 papads in one poly bag. Store in a cool and dry place.

Note: To consume the product, it has to be fried at 170°C for 60 to 90 sec in cooking oil.

8. Mushroom Pakoda

Ingredients: According to Markam (2014) following is required for preparing mushroom pakoda.

Table: List of ingredients used for the preparation of mushroom Pakoda

1.	Fresh mushrooms	500 g (Button or oyster or milky)
2.	Gram flour (besan)	150 g
3.	Onion	1 big (chopped)
4.	Ginger	2 tbl (chopped)
5.	Garam masala	10 g
6.	Anar dana powder	1 tbl.
7.	Cooking oil	100 g
8.	Salt, green chillies (chopped)	to taste

Method

Before using the mushrooms to make pakoda, it is a good idea to blanch them. The mushrooms should be boiled for five minutes in hot, salted water. After allowing the mushrooms to drain, cut them up, and combine them with the other ingredients mentioned above. Mix thoroughly. Make a gramme flour mixture and include salt and pepper in it as well. Form mixture into tiny balls, coat with besan, and fry until golden. Serve warm with sauce or chutney.

We are able to create a variety of other

pakodas. These involve using small mushrooms by themselves and using mushroom caps that have been filled with cheese or palak and then joined together using toothpicks (mushroom duplex). By hand or with a knife, cut 100g of oyster mushrooms into small pieces. Add a pinch of salt, pepper, etc., as well as one or two spoons of besan. By hand, combine it. Water doesn't need to be added. To obtain crisp pakoda, we can fry these items in a hot pan until golden brown.

9. Mushroom-Based Cosmetics

Utilizing the advantageous qualities of mushrooms, skincare and haircare products are made from a mushroom base. Polysaccharides, antioxidants, and vitamins are among the substances found in mushrooms that nourish and shield the skin and hair. The following procedures are typically involved in the production of cosmetics made from mushrooms:

- **Raw Material Preparation**: Those mushrooms that have the needed cosmetic qualities are chosen and prepared. For the purpose of getting the active compounds, they can be dried, ground into a powder, or extracted.
- **Extraction**: The desired bioactive compounds are extracted from the mushrooms, if necessary, using the appropriate solvents. Different extraction techniques, such as solvent extraction, infusion, and maceration, can be used.
- **Formulation**: Creams, lotions, serums, shampoos and conditioners are just a few examples of cosmetic products that contain extracts from mushrooms or mushroom powder. The finished product is made up of various extra substances including emulsifiers, preservatives and fragrances.
- **Testing and Quality Control**: To make guaranteed the cosmetics are safe, stable, and effective, testing and quality control procedures are used. The efficacy and shelf life of the product is verified through microbiological and stability checks.
- **Packaging**: The mushroom-based cosmetics are packaged in suitable containers, ensuring hygiene, protection from light and ease of use.

Preservation of Mushrooms

By soaking the mushrooms in a solution of acids or salts, you are able to store them for even a brief period of time using this easy and affordable approach. To preserve blanched mushrooms, a solution made up of 2.0 per cent sodium chloride, 2.0 per cent citric acid, 2.0 per cent sodium bicarbonate, and 0.15 per cent KMS is steeped for 8-10 days at 21-28 °C.

Conclusion

Technology used in mushroom production includes a number of methods and procedures that are crucial for effective mushroom culture. Each phase is essential for optimizing efficiency and maintaining the quality of the harvested mushrooms, from substrate preparation to spawn production, growing methods and environmental control. Farmers and growers can fulfil the rising demand for mushrooms and contribute to the sustainable production of this important crop by employing suitable mushroom cultivation technologies.

Mushroom-derived value-added products have several uses and advantages across numerous fields. Just a few examples of the numerous product possibilities include mushroom extracts, mushroom powder, and mushroom-based cosmetics. Utilizing mushroom resources to the fullest extent requires an understanding of the industrial procedures used to produce these value-added products. We can take advantage of the nutritional and therapeutic features of mushrooms by integrating them into a lot of businesses which will help us build new, sustainable goods.

References

Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., Junior A. M. and Ribani, R. H. (2019). Factors affecting mushroom *Pleurotus* spp. *Saudi Journal of Biological Sciences*, **26**(4): 633-646. (http://dx.doi.org/10.1016/j.sjbs.2016.12.005)

BordGlas. (2002). In: The Horticultural Development Board (ed.). Mushrooms. Dublin, Ireland: BordGlas. 25pp.

Cliffe-Byrnes, V. A. L. E. R. I. E. and O'beirne, D. (2010). Process-Modified Atmosphere and Humidity Parameters for High-Quality Sliced Mushrooms (*Agaricus bisporus* L.). *Journal of Food Quality*, **33**, 286-302.

Cunha Zied, D., Sánchez, J. E., Noble, R. and Pardo-Giménez, A. (2020). Use of spent mushroom substrate in new mushroom crops to promote the transition towards a circular economy. *Agronomy*, **10**(9), 1239.

Gowda, N. A. and Manvi, D. (2019). Agro-residues disinfection methods for mushroom cultivation. *Agricultural Reviews*, **40**(2): 93-103.

Mahajan, P. V., Oliveira, F. A. R. and Macedo, I. (2008). Effect of temperature and humidity on the transpiration rate of the whole mushrooms. *Journal of Food Engineering*, **84**(2): 281-288.

Mohd Hanafi, F. H.; Rezania, S.; Mat Taib, S.; Md Din, M. F.; Yamauchi, M.; Sakamoto, M.; Hara H.; Park, J. and Ebrahimi, S. S. (2018). Environmentally

sustainable applications of agro-based spent mushroom substrate (SMS): an overview. *Journal of Material Cycles and Waste Management*, **20**: 1383-1396.

Pandey, M., Satisha, G. C., Azeez, S., Kumaran, G. S. and Chandrashekara, C. (2022). Mushrooms for integrated and diversified nutrition. *Journal of Horticultural Sciences*, **17**(1): 6-18.

Sahoo, S., Gayakwad, T. and Shahi, S. (2022). Medicinal value of edible mushrooms: A review. *International Journal of Health Sciences*, (2): 8760-8767.

Wakchaure, G. C. (2011). Powder, A. M. S. Mushrooms-Value Added Products. Directorate of Mushroom Research, Solan, Himachal Pradesh. pp. 233-238. (Available on: https://www.researchgate.net/publication/235957071_Mushrooms-Value_Added_Products)

Markam, S. (2014). Value addition of mushroom. *Journal of Pharmacognosy and Phytochemistry*, **3**(4): 253-256.

13 Ecological Engineering in Plant Disease Management

Jyoti Kumari[1], Ankit Kumar Singh[2], Puja Kumari[3] and Kumar Aditya[4]

[1]Ph.D. Scholar, Deptt. of Plant Pathology, RAC, BAU, Kanke, Ranchi, JH
[2]Ph.D. Scholar, Deptt. of Entomology, RAC, BAU, Kanke, Ranchi, JH
[3]Ph.D. Scholar, Deptt. of Plant Pathology, BAU, Sabour, Bihar
[4]Ph.D. Scholar, Deptt. of Plant Pathology, IAS, BHU, Varanasi, U.P.

Abstract

Ecological engineering, an innovative and sustainable approach, has gained increasing attention in the realm of plant disease management. This paper explores the concept of ecological engineering in the context of plant disease control, highlighting its key principles and techniques. Ecological engineering strategies aim to enhance ecosystem resilience, functional diversity, and natural control mechanisms to mitigate disease outbreaks. The role of biodiversity in providing ecosystem services, such as biological control and disease suppression, is a central aspect of ecological engineering (Cook & Baker, 1983). By manipulating habitat structure, fostering beneficial interactions, and promoting biological control agents, ecological engineering effectively contributes to sustainable plant disease management.

Keywords: Ecological engineering, plant disease management, biodiversity, habitat manipulation, biological control, ecosystem services, sustainable agriculture.

Introduction

Global food security and agricultural output are seriously threatened by plant diseases. Traditional disease management strategies frequently rely on the application of chemical pesticides, which can be harmful to the environment and human health (Bélanger *et al.,* 2003). Creating alternative and sustainable plant disease control methods has garnered more attention in recent years. By incorporating ecological

concepts into disease management strategies, the multidisciplinary discipline of ecological engineering offers promising answers.

The focus of ecological engineering is on modifying ecosystem functions to improve plant health and prevent the spread of disease. This method seeks to create a balance that lowers the incidence of disease while acknowledging the interconnectivity of plants, pests, and diseases within agroecosystems. Ecological engineering encourages sustainable disease management techniques with a low reliance on synthetic inputs by utilising ecological interactions.

Several important guiding concepts govern ecological engineering in the management of plant diseases. First, it places a focus on the promotion of biodiversity since diverse plant communities frequently have higher disease resistance. The second area of ecological engineering is the development of biological controls and natural enemies that inhibit plant diseases. Thirdly, it emphasises the significance of microbial variety and soil health, as these factors help to prevent disease. Finally, cultural practices that make it difficult for pathogens to develop and grow are supported by ecological engineering.

Application of Ecological Engineering for Plant Disease Management

- **Biological Control Agents:** The use of helpful microbes and natural enemies for disease prevention is one of the main strategies in ecological engineering. Plant infections can be successfully controlled by biocontrol agents like specific fungus, bacteria, and insects through competition, predation, or antagonism. Utilising these compounds in disease management plans can drastically cut back on the use of chemical pesticides (Matsui & Cowling, 2004).
- **Habitat Manipulation:** An additional important component of ecological engineering is the manipulation of ecosystems to produce a variety of favourable settings for natural enemies (Kessel *et al.,* 2004). For instance, adding flowering plants might draw beneficial insects that serve as parasitoids or predators of plant infections.
- **Cultural Practices:** In order to manage disease, ecological engineering must take cultural practises into account. Crop rotation, intercropping, and mixed cropping are a few techniques that can stop the growth of pathogen populations and stop the spread of disease.

Basic Principles of Ecological Engineering for Plant Disease Control

Plant diseases present serious obstacles to global agricultural productivity. Chemical

pesticides, which can be harmful to the environment and human health, are frequently used in traditional ways to disease management. By utilising ecological principles to control plant diseases in a sustainable and environmentally responsible way, ecological engineering presents an alternative paradigm.

- **Biodiversity and Disease Suppression:** In ecological engineering for plant disease prevention, biodiversity is essential. Through a variety of mechanisms, such as dilution effects, where a large diversity of plant species lowers the possibility of pathogen establishment and dissemination, diverse plant communities can demonstrate greater resistance to diseases (Van Elsas *et al.,* 2012). Additionally, a variety of natural enemies and advantageous bacteria that help to reduce disease are supported by various habitats. With the help of techniques like crop rotation, intercropping, and agroforestry, ecological engineering can significantly lower the prevalence of illness.
- **Biological Control:** A key component of ecological engineering for plant disease management is biological control. It entails using predatory insects, parasitic fungus, and bacteria as plant diseases' natural adversaries to control disease outbreaks. These biological pesticides have the ability to kill infections directly or make plants develop systemic resistance. Ecological engineering strengthens the natural processes of biological control, minimising the need for chemical pesticides, through the preservation and enhancement of natural enemies and the strategic release of beneficial organisms.
- **Habitat Manipulation:** Another key element of ecological engineering for plant disease prevention is habitat manipulation. The natural enemies of plant infections can be attracted to and maintained in agroecosystems by altering the agricultural environment to create optimal habitats for helpful species. For instance, adding flowering plants or creating hedgerows might offer resources like nectar and pollen, luring predatory insects and parasitic wasps. Implementing cover crops and developing refuge zones can give natural enemies a home and a place to reproduce, aiding in the effective management of disease.
- **Cultural Practices:** In order to control plant diseases, ecological engineering makes substantial use of cultural practises. It is feasible to create an environment that is unfavourable for pathogen development and growth by implementing appropriate agronomic practises. For instance, crop rotation breaks up disease cycles by avoiding the accumulation of pathogen populations. By limiting the number of susceptible hosts, intercropping and mixed cropping can reduce the geographical variety that allows disease to spread. In addition to

promoting plant health and bolstering their built-in defences against disease, optimal irrigation and nutrient management techniques do the same.

» **Integrated Pest Management (IPM):** The principles of ecological engineering can be successfully incorporated into Integrated Pest Management (IPM) techniques. IPM emphasises a comprehensive and environmentally friendly approach to managing pests and diseases, incorporating several control strategies, such as biological, cultural, and chemical techniques. Enhancing disease control effectiveness while reducing environmental risks is attainable by adding ecological engineering ideas into IPM programmes (Steinberg & Edel-Hermann, 2019).

Role of Biodiversity in Plant Disease Management

A given ecosystem's biodiversity refers to the range of living things, such as plants, animals, and microorganisms. It is a crucial element of robust and healthy ecosystems and is essential to many ecosystem functions. Biodiversity has been acknowledged as a key component influencing disease dynamics and control techniques in the context of plant disease management.

Biodiversity and Disease Suppression

» **Dilution Effect:** The dilution effect is one important way that biodiversity promotes illness prevention. The presence of non-host or less vulnerable plant species in varied plant communities might limit the number of available hosts for diseases, hence lowering the prevalence of disease. In monoculture settings, where a single host species creates an ideal environment for pathogen development, the dilution impact can be very significant.

» **Increased Genetic Diversity:** Increased genetic diversity among plant populations can improve disease resistance. The presence of people with various levels of susceptibility due to genetic variation lessens the overall burden of disease on the population (Poveda & Rausher, 2011). The development of many plant defence mechanisms, such as the creation of secondary metabolites or the activation of systemic acquired resistance (SAR), which can give resistance against a variety of diseases, is also encouraged by genetic diversity.

» **Trophic Interactions and Biological Control:** Through trophic interactions and the encouragement of biological control, biodiversity can have an impact on plant disease management. A range of natural enemies, such as predators, parasitoids, and entomopathogens, can live in many ecosystems and control the populations of plant pathogens by preying on or parasitizing them.

These innate antagonists have the power to exercise top-down control over pathogen populations and lower the occurrence of disease.

» **Soil Microbial Diversity and Disease Suppression:** A crucial aspect of biodiversity that significantly contributes to the prevention of disease is soil microbial diversity. Through a variety of methods, such as competition for resources and space, the production of antimicrobial substances, and the induction of systemic resistance in plants, diverse microbial communities in the soil can inhibit the growth of pathogens. In addition, some types of microbes can actively compete with plant pathogens, serving as biocontrol agents.

» **Implications for Agricultural Practices:** The control of plant diseases will be impacted significantly by our understanding of biodiversity. The following actions can support disease prevention and biodiversity enhancement:

» **Crop Rotation:** Over time, different crop species are systematically alternated in the same field. By forcing times during which the diseases come into contact with non-host or less vulnerable plant species, it prevents the growth of pathogen populations. Crop rotation increases biodiversity, lowers the likelihood of illness, and boosts the general health of the crops.

» **Polyculture and intercropping:** Growing many crop species simultaneously in the same field is known as polyculture and intercropping. These methods boost biodiversity, provide diseases a more complex environment to live in, and stop their spread. Intercropping can improve disease control by combining crop species with complimentary growth patterns or disease resistance features.

» **Agroforestry and Hedgerows:** By incorporating trees and shrubs into agricultural landscapes through agroforestry or hedgerows, it is possible to support natural enemies of plant pathogens and considerably increase biodiversity. In order to increase the populations of beneficial insects and their efficiency in eradicating diseases, trees and shrubs offer shelter, nectar sources, and alternate prey to these insects.

» **Biological control for conservation:** Biological control for conservation entails the creation and maintenance of habitats that sustain beneficial creatures, such as flowering plants for pollinators and natural enemies. Hedgerows, areas of wildflowers, and cover crops are a few examples of these environments. Conservation biological control improves disease suppression by fostering the diversity and abundance of helpful organisms.

Suppression of Plant Disease by Biological Control Agents

Significant obstacles to agricultural productivity and food security are posed by plant diseases. Chemical pesticides, which can have negative impacts on the environment, human health, and beneficial creatures, are frequently used in traditional disease management tactics. A different strategy is provided by biological control, which uses plant diseases' natural enemies to control disease outbreaks. This article focuses on the potential of biological control agents for sustainable agriculture as well as their role in the suppression of plant diseases.

Mechanism of Action: To combat plant diseases, biological control agents use a variety of ways. These mechanisms, which may be direct or indirect, consist of:

1. **Predation:** Plant pathogens are eaten by predatory insects including ladybirds, lacewings, and predatory mites, which lowers their numbers and the severity of illness. These predators actively hunt for and eat infections, upsetting their life cycles and stopping the spread of disease.
2. **Parasitism:** Infecting and consuming plant diseases, parasitic organisms like parasitic fungus and parasitic wasps eventually kill them. These biological control agents parasitize diseases by either attacking them directly and eating them or by depositing eggs inside of them, which eventually kills the pathogens.
3. **Antagonism:** Some bacteria and fungi create antimicrobial substances that prevent plant pathogens from growing and acting. These biocontrol agents produce chemicals that either directly limit pathogen development or directly kill it while competing with pathogens for nutrition and space.
4. **Induced Resistance:** Some biological pesticides can make plants more disease-resistant by inducing systemic resistance (Ferrandino & Smart, 2017). In order to ward off pathogen attacks, this process involves the activation of plant defense mechanisms, such as the creation of antibacterial chemicals or the reinforcing of cell walls.

Application Techniques: Depending on the type of biological control agent and the target disease, various application techniques can be used. Typical application techniques include:

» **Inundative Release:** Inundative release entails the widespread dispersal of advantageous organisms in order to immediately establish their populations and exert instant control on the target diseases. When disease outbreaks are about to occur or when pathogen populations are already high, this technique is frequently employed.

- **Inoculative Release:** To maintain a steady population that can continuously control disease, inoculative release entails the periodic release of helpful organisms. This approach is frequently used for the long-term control and prevention of disease.
- **Conservation:** The goal of biological control through conservation is to establish and preserve ecosystems that are favourable to the survival of natural enemies. Giving beneficial creatures a place to live, access to food supplies, and appropriate habitats promotes their presence and helps them naturally control plant diseases.

Biological control's advantages for sustainable agriculture

- **Less Reliance on Chemical Pesticides:** Using biological control agents lessens the need for chemical pesticides, reducing the dangers connected to their usage, such as environmental contamination, issues with human health, and the emergence of pesticide resistance.
- **Targeted Control:** Biological control agents can specifically target the pathogens of interest, minimising unintended effects on beneficial organisms and maintaining the ecosystems' natural balance.
- **Application in IPM:** Biological control agents can be used into integrated pest management (IPM) programmes, which combine several control strategies for long-term pest and disease management (Jørgensen *et al.,* 2017). Synergistic effects can be produced by adding biological control agents into IPM techniques, resulting in more efficient and long-lasting disease control.
- **Long-Term Efficacy:** Biological control agents can build up populations and last in the environment, preventing disease for a considerable amount of time. This persistence improves the sustainability of disease management practises by reducing the requirement for recurrent administrations.

Using Beneficial Microorganisms to Manage Disease

Significant risks to agricultural productivity and food security are posed by plant diseases. Chemical pesticides, which can have negative impacts on ecosystems, non-target creatures, and human health, are frequently used in traditional disease management tactics. A green method of managing disease is to use advantageous microorganisms as biocontrol agents. Through a variety of ways, these bacteria interact with plant pathogens to restrict the spread of disease and improve plant health.

Mechanism of Action: To control plant diseases, helpful microbes use a variety of processes, including:

- **Competition:** For resources like nutrients and space, beneficial microbes may compete with plant diseases, which could restrict their growth and establishment. These microbes prevent pathogens from colonising plant tissues or surfaces by out-competing them, which lowers the prevalence of disease.
- **Antagonism:** Some helpful microbes create antimicrobial substances, including enzymes or antibiotics, that prevent the development and activity of plant diseases. These substances damage the cell walls of pathogens, obstruct their metabolism, or cause cell lysis, which kills or suppresses the pathogen.
- **Induced Systemic Resistance:** ISR, also known as induced systemic resistance (ISR), is a condition where beneficial bacteria increase the plant's immune system. Plants become more resistant to successive pathogen attacks by activating defence mechanisms through ISR, such as the production of antimicrobial compounds, pathogenesis-related proteins, and phytohormones.
- **Nutrient Enhancement:** Some advantageous microbes can increase the availability and uptake of nutrients for plants, enhancing their health and vigour. By encouraging healthier plants that are better able to survive pathogen attacks, this covert mechanism influences disease management covertly.

Application Techniques: Depending on the type of microbe and the target pathogen, beneficial microorganisms can be applied using a variety of techniques. Typical application techniques include:

- **Treatment of seeds:** Before planting, treating seeds with helpful microbes ensures their colonisation and establishment within the sprouting seedlings. This technique encourages plant growth right away and offers early defence against soilborne diseases (Lefèvre & Fravel, 2020).
- **Foliar Sprays:** Foliar sprays are used to introduce advantageous microorganisms directly to plant leaves. This technique protects against foliar illnesses and enables microorganisms to colonise the leaf surface and interact with plant pathogens.
- **Soil Drench:** A soil drench is the application of advantageous microorganisms to the root zone so that they can flourish and engage in interactions with soilborne diseases (Malvick, 2016). This approach guards against illnesses spread through dirt and enhances nutrient availability for plants.

» **Inoculant Application:** To the planting media or soil, beneficial microorganisms can be added as inoculants. High quantities of the helpful microorganisms are present in inoculants, which are employed to establish their populations in the target environment to permanently decrease disease.

Advantages of using advantageous microorganisms for disease management

» **Environmentally friendly:** Beneficial microorganisms provide environmentally benign substitutes for chemical pesticides, reducing the dangers connected with chemical use, such as soil and water contamination, toxicity to organisms that aren't the target, and the emergence of pesticide resistance.

» **Sustainable Agriculture:** The use of advantageous microorganisms encourages sustainable farming practises by minimising dependency on chemical inputs. By lowering the frequency of pesticide treatments and enhancing long-term soil health, these microorganisms aid in the development of resilient agricultural systems.

» **Integrated Pest Management (IPM):** IPM strategies, which combine several control techniques for sustainable pest and disease management, can incorporate the use of beneficial microbes. Integrated strategies improve disease management programmes' efficacy while lessening their negative effects on the environment.

» **Broad Spectrum of action:** Helpful microorganisms can show a wide range of action against numerous plant pathogens, controlling several illnesses at once. They are useful instruments for managing a variety of disease complexes in agricultural systems because of their adaptability.

Enhancing Soil Health for Disease Suppression

Soil-borne diseases are a major problem for agricultural productivity. Traditional approaches to disease management often rely on chemical pesticides, which can have negative environmental and health impacts. However, there is a growing body of evidence that suggests that enhancing soil health can be an effective and sustainable way to suppress soil-borne diseases.

Healthy soils contain a diverse range of beneficial microorganisms, including bacteria, fungi, and viruses. These microorganisms play a number of important roles in disease suppression, including:

» **Direct antagonism:** Beneficial microorganisms can directly antagonize plant pathogens by competing for resources, producing antimicrobial compounds, or parasitizing the pathogens.

- **Induced systemic resistance (ISR):** Healthy soils can induce ISR in plants, which primes them for enhanced defense against pathogens. ISR is a plant defense mechanism that is triggered by the presence of beneficial microorganisms.
- **Nutrient competition:** Beneficial microorganisms can compete with pathogens for nutrients, limiting the resources available for pathogen growth and suppressing disease development.
- **Soil structure and water management:** Optimal soil structure and water management practices promote root health and vigor, improving plant resilience to diseases. Well-drained soils with good water-holding capacity reduce the risk of waterlogged conditions, which can favor certain soil-borne pathogens.

Strategies for Enhancing Soil Health for Disease Suppression:

- **Organic matter management:** Incorporating organic amendments, such as compost or cover crops, enhances soil organic matter content, nutrient availability, and microbial activity. These practices improve soil structure, water retention, and nutrient cycling, leading to healthier soils and enhanced disease suppression.
- **Crop rotation and diversification:** Implementing crop rotation and diversification practices disrupts disease cycles, reduces pathogen buildup, and enhances soil microbial diversity. Different crop species have varying susceptibilities to diseases, and alternating crops can break disease cycles, limiting the spread and establishment of soil-borne pathogens.
- **Biological soil amendments:** Applying beneficial microorganisms, such as biocontrol agents or microbial inoculants, can introduce or enhance the populations of disease-suppressive organisms in the soil. These amendments promote disease suppression through antagonism, induced systemic resistance, and nutrient competition.
- **Proper nutrient management:** Balanced and targeted nutrient management ensures optimal nutrient availability for plants and supports disease suppression. Regular soil testing and tailored nutrient application based on crop needs can help maintain nutrient balance and prevent nutrient imbalances that can predispose plants to diseases.

Plant Disease Management through Habitat Manipulation

The goal of habitat manipulation is to change the agricultural environment to support beneficial species and reduce plant diseases. The natural biological control of diseases is strengthened and overall plant health is promoted through habitat manipulation, which modifies the structure, variety, and resource availability of the environment.

Principles of Habitat Manipulation for Disease Management

- **Biodiversity:** Increasing biodiversity within the agroecosystem is a fundamental principle of habitat manipulation. Diverse habitats support a wide range of organisms, including natural enemies of plant pathogens, which can provide biological control. Higher biodiversity enhances the stability and resilience of the ecosystem, making it more resistant to disease outbreaks.
- **Connectivity:** Creating habitat patches or corridors that connect different habitats within the landscape facilitates the movement of beneficial organisms, such as pollinators, predators, and parasitoids. Increased connectivity improves their access to resources and target areas, enhancing their effectiveness in suppressing diseases.
- **Resource Provision:** Habitat manipulation involves providing suitable resources, such as floral resources for beneficial insects or nesting sites for natural enemies, within or near agricultural fields. These resources attract and support beneficial organisms, increasing their abundance and promoting their activity against plant pathogens.
- **Planting floral resources:** Incorporating flowering plants in and around agricultural fields provides nectar and pollen resources for beneficial insects, including predators and parasitoids. These insects can feed on floral resources and subsequently prey on or parasitize plant pathogens, contributing to disease suppression.
- **Implementing cover crops:** Cover crops serve as temporary habitat and provide multiple benefits for disease management. They enhance soil health, suppress weeds, and attract beneficial insects. Cover crops with diverse plant species promote biodiversity, enhance natural enemy populations, and limit the spread of diseases.
- **Establishing hedgerows and windbreaks:** Hedgerows and windbreaks act as barriers or borders around agricultural fields, creating diverse habitats that support beneficial organisms. These linear features provide shelter, nesting sites, and floral resources, attracting natural enemies of plant pathogens and enhancing their presence in the agroecosystem.

- **Maintaining riparian zones and wetlands:** Preserving riparian zones and wetlands within or adjacent to agricultural areas enhances habitat diversity and water availability. These habitats promote the presence of natural enemies and can act as buffers, reducing the movement of pathogens from water sources into agricultural fields.

Benefits of habitat manipulation for disease management

- **Enhanced biological control:** Habitat manipulation promotes the abundance and activity of natural enemies, such as predatory insects, parasitoids, and insectivorous birds, which contribute to biological control (Mäntylä *et al.,* 2017). Increased populations of these beneficial organisms can lead to efficient and sustainable disease suppression.
- **Reduced pesticide use:** Effective habitat manipulation strategies can reduce the reliance on chemical pesticides. By enhancing natural biological control, habitat manipulation decreases the need for chemical interventions, minimizing environmental risks and preserving ecosystem health.
- **Enhanced pollination:** Habitat manipulation practices that promote floral resources and attract pollinators contribute to improved pollination services. Adequate pollination enhances crop yields, quality, and resilience against diseases.
- **Ecosystem services:** Habitat manipulation provides additional ecosystem services, such as improved soil health, water retention, and carbon sequestration. These services contribute to the overall sustainability and resilience of agricultural systems.

Plant Disease Management through cultural practices

Cultural practices are essential components of ecological disease management, emphasizing the modification of cultural practices to prevent disease outbreaks and promote plant health (Vidal & Kikkert, 2010). Unlike chemical interventions, cultural practices focus on sustainable approaches that minimize environmental impacts and promote long-term disease suppression.

Key benefits of cultural practices for ecological disease management:

- **Sustainable and environmentally friendly:** Cultural practices offer sustainable and environmentally friendly approaches to disease management. By emphasizing prevention, these practices reduce reliance on chemical interventions, minimizing environmental risks and preserving ecosystem health.

- **Long-term disease suppression:** Cultural practices contribute to long-term disease suppression by targeting the underlying factors that influence disease development. These practices promote soil health, biodiversity, and crop resilience, creating an agroecosystem that is less susceptible to diseases over time.
- **Integrated Pest Management (IPM):** Cultural practices can be integrated into IPM programs, combining multiple control tactics for sustainable disease management. They complement other IPM components, such as biological control and chemical interventions, to enhance disease suppression while minimizing environmental impacts.
- **Cost-effective:** Cultural practices are often cost-effective compared to chemical interventions. They focus on preventative measures, reducing the need for expensive inputs and frequent pesticide applications. By implementing cultural practices, growers can optimize resource utilization and minimize economic losses due to diseases.

Specific cultural practices that can be used for ecological disease management:

1. **Crop rotation:** Crop rotation is a fundamental cultural practice that involves the systematic alternation of different crop species within a specific field or across the farm. This practice disrupts the life cycles of plant pathogens, preventing the buildup of pathogen populations and reducing disease incidence.
2. **Sanitation:** Sanitation practices focus on the removal and destruction of plant debris, infected plants, and diseased materials. Prompt removal of infected plant parts reduces the availability of inoculum sources, limiting disease spread. Proper disposal of diseased materials through burning, burying, or composting helps prevent the survival and spread of pathogens. Sanitation practices also include cleaning and disinfecting tools, equipment, and greenhouse structures to minimize the transmission of pathogens.
3. **Planting density and spacing:** Planting density and spacing are critical factors in disease management. Proper plant spacing promotes good airflow and light penetration, reducing humidity levels and creating a less favorable environment for disease development. Adequate spacing between plants prevents the spread of pathogens through direct contact and improves the efficacy of cultural practices, such as pruning and spraying. Optimal planting density and spacing ensure that plants have adequate access to light, nutrients, and water, promoting overall plant health and disease resistance.

4. **Timing of operations:** Timing of cultural practices, such as planting, irrigation, fertilization, and harvesting, plays a crucial role in disease management. Timely operations can help minimize disease risks by avoiding periods of high pathogen activity and vulnerability of plants. For example, planting during optimal weather conditions can promote vigorous growth and reduce susceptibility to diseases. Similarly, irrigation and fertilization practices should be tailored to avoid creating favorable conditions for pathogen growth and spread.
5. **Soil health management:** Healthy soils are essential for plant disease management. Cultural practices that promote soil health, such as organic matter management, cover cropping, and minimizing soil disturbance, contribute to disease suppression. Organic matter enhances soil fertility, microbial diversity, and nutrient availability, promoting overall plant health and resilience to diseases. Cover cropping improves soil structure, water infiltration, and nutrient cycling, creating an environment conducive to beneficial microorganisms and suppressing pathogens.

Interactions between plants, pests, and diseases in agroecosystems

Agroecosystems are complex and dynamic systems consisting of crops, pests, diseases, and their environment. Plants, pests, and diseases interact within these systems, creating intricate ecological relationships that impact agricultural productivity and sustainability. Understanding these interactions is essential for developing effective pest and disease management strategies that minimize environmental impacts and promote sustainable agriculture (Tamm *et al.,* 2018).

Factors Influencing Interactions:

Several factors influence the interactions between plants, pests, and diseases in agroecosystems:

- **Plant Traits:** Plant traits, such as genetic composition, growth habits, and physiological characteristics, influence their susceptibility or resistance to pests and diseases. Some plants may possess natural defenses, such as secondary metabolites or physical barriers, that deter pests or limit disease development.
- **Pest and Disease Dynamics:** Pest and disease populations are influenced by various factors, including environmental conditions, host availability, and natural enemies. Changes in these factors can affect pest and disease abundance, distribution, and severity within agroecosystems.

» **Environmental Conditions:** Environmental factors, such as temperature, humidity, and precipitation, influence the development and spread of pests and diseases. Favorable environmental conditions can accelerate pathogen growth, insect reproduction, and disease progression, leading to increased risks and impacts.

» **Landscape Structure:** The structure and composition of the surrounding landscape can influence pest and disease dynamics within agroecosystems. Landscape features, such as nearby forests, water bodies, or non-crop vegetation, can serve as reservoirs for pests or pathogens, contributing to their spread into agricultural fields.

Direct Interactions:

» **Plant-Pest Interactions:** Pests, including insects, mites, nematodes, and rodents, interact directly with plants, causing damage through feeding, tunneling, or oviposition (Müller & Hilker, 2017). These interactions can lead to yield losses, reduced quality, and increased susceptibility to diseases.

» **Plant-Disease Interactions:** Pathogens, including bacteria, fungi, viruses, and nematodes, interact directly with plants, causing infections and diseases (Thomas & Blanford, 2003). Pathogens can invade plant tissues, reproduce, and spread, leading to symptoms such as wilting, necrosis, or discoloration.

Indirect Interactions:

» **Plant-Pest-Disease Interactions:** Pests and diseases can interact indirectly through their effects on plants. For example, pest damage, such as feeding or tunneling, can create entry points for pathogens, increasing the susceptibility of plants to diseases. Similarly, diseases can weaken plants, making them more susceptible to pest attacks.

» **Plant-Microbe Interactions:** Beneficial microorganisms, such as rhizobacteria or mycorrhizal fungi, can interact with plants and influence their resistance to pests and diseases (Liang *et al.,* 2018). These microorganisms can induce systemic resistance or promote nutrient uptake, enhancing plant health and resilience against pests and pathogens.

Implications for Sustainable Agriculture:

Understanding the interactions between plants, pests, and diseases in agroecosystems is crucial for sustainable agriculture:

» **Integrated Pest Management (IPM):** Integrated approaches that consider

the complex interactions within agroecosystems are essential for effective pest and disease management. Integrated Pest Management (IPM) combines multiple control tactics, such as cultural practices, biological control, and chemical interventions, to minimize pest and disease impacts while minimizing environmental risks.

» **Crop Diversity:** Promoting crop diversity within agroecosystems can disrupt pest and disease cycles and reduce the risk of severe outbreaks. Growing different crop species or varieties with varying growth habits and disease resistance traits can create a less favorable environment for pests and diseases, minimizing their impacts.

» **Habitat Manipulation:** Manipulating the habitat structure within and around agroecosystems can enhance biological control and promote plant health. Creating diverse habitats, such as hedgerows or cover crops, provides shelter, nectar resources, and alternative prey for beneficial insects, promoting their abundance and effectiveness in suppressing pests and diseases.

» **Cultural Practices:** Implementing cultural practices, such as crop rotation, sanitation, and optimal planting density, can reduce pest and disease pressures. These practices disrupt pest and disease life cycles, remove disease sources, and create conditions less favorable for pathogen development and pest establishment

Utilizing Plant Diversity for Disease Resistance

Plant diseases pose significant threats to agricultural productivity and food security. Utilizing plant diversity offers a sustainable approach to disease resistance by harnessing the natural defense mechanisms present in different plant species and varieties.

» **Importance of Genetic Diversity:** Genetic diversity within plant populations provides a valuable resource for disease resistance. Different plant genotypes exhibit varying degrees of resistance to pathogens, allowing for the selection of resistant individuals or varieties. Genetic diversity increases the likelihood of encountering resistant genotypes and enables the development of disease-resistant crops through breeding programs.

» **Role of Plant Secondary Metabolites:** Plant secondary metabolites, such as phytoalexins, alkaloids, and phenolic compounds, play a crucial role in disease resistance. These compounds are produced in response to pathogen attacks and can inhibit pathogen growth, disrupt their life cycles, or strengthen plant defense mechanisms. Plant diversity provides a wide range of secondary

metabolites with different chemical properties, enhancing the potential for disease resistance.

» **Utilizing Resistant Cultivars:** Selecting and utilizing resistant cultivars is a practical approach for disease management. Resistant cultivars are developed through breeding programs that aim to incorporate disease resistance genes into elite plant varieties. These cultivars exhibit improved resistance to specific diseases, reducing the need for chemical interventions and providing sustainable disease management solutions.

» **Exploiting Wild Relatives:** Wild relatives of cultivated plants are valuable sources of genetic diversity and disease resistance traits. These species have evolved under diverse environmental conditions and have developed robust defense mechanisms against pathogens. By incorporating genes from wild relatives into cultivated varieties, breeders can enhance disease resistance and develop crops with improved resilience to diseases.

Enhancing Plant Diversity in Agricultural Systems:

There are a number of ways to enhance plant diversity in agricultural systems, including:

» **Crop rotation:** Crop rotation involves the systematic alternation of different crop species in a specific field or across the farm. Rotating crops with varying disease susceptibilities disrupts disease cycles and reduces the buildup of pathogen populations. Crop rotation increases genetic diversity within the agroecosystem, creating conditions less favorable for disease development and promoting overall disease resistance.

» **Intercropping:** Intercropping involves growing multiple crops in close proximity within the same field. Intercropping promotes biodiversity, enhances ecological interactions, and reduces disease incidence. The diverse plant species in intercropping systems can interfere with pathogen dispersal, provide natural barriers, and improve overall disease resistance through the dilution effect.

» **Agroforestry systems:** Agroforestry systems integrate trees with agricultural crops, creating diverse and dynamic landscapes. These systems enhance biodiversity, provide microclimatic conditions, and promote beneficial interactions among plants. Agroforestry systems contribute to disease resistance by enhancing habitat diversity, attracting beneficial organisms, and creating a more resilient agroecosystem.

Benefits of Utilizing Plant Diversity for Disease Resistance

There are a number of benefits to utilizing plant diversity for disease resistance, including:

- **Sustainable disease management:** Utilizing plant diversity for disease resistance reduces the reliance on chemical interventions and promotes sustainable disease management practices. Genetic diversity and resistance traits contribute to long-term disease suppression, improving crop health and reducing economic losses.
- **Environmental protection:** Maximizing plant diversity in agricultural systems contributes to environmental protection. By reducing the use of chemical pesticides and promoting ecosystem resilience, plant diversity helps maintain biodiversity, preserve beneficial organisms, and minimize the environmental impacts associated with disease management.
- **Resilience to changing conditions:** Plant diversity enhances the resilience of agricultural systems to changing environmental conditions and emerging diseases (Altieri & Nicholls, 2013). The wide range of genetic diversity and defense mechanisms present in diverse plant populations provides a reservoir of traits that can adapt to new challenges and stresses.

Sustainable Management Strategies Using Ecological Engineering

Ecological engineering is an interdisciplinary field that applies ecological principles to design and manage ecosystems for sustainable outcomes (Gurr *et al.,* 2004). It encompasses the integration of biological, physical, and engineering concepts to create harmonious and resilient systems.

Key Principles of Ecological Engineering

- **Functional diversity:** Ecological engineering focuses on enhancing functional diversity within ecosystems. Functional diversity refers to the variety of species and ecological roles present in a system. By promoting diverse functional groups, ecological engineering aims to improve ecosystem stability, resilience, and productivity.
- **Ecosystem services:** Ecological engineering aims to enhance the provision of ecosystem services, which are the benefits that ecosystems provide to humans. These services include pollination, soil fertility, water purification, pest control, and climate regulation. By optimizing ecosystem functions, ecological engineering supports the sustainable provision of these services.
- **Natural processes and feedbacks:** Ecological engineering emphasizes

harnessing and enhancing natural processes and feedbacks within ecosystems. Understanding and leveraging natural interactions, such as nutrient cycling, predator-prey relationships, and plant-soil interactions, can lead to more sustainable and self-regulating systems.

Techniques and Strategies of Ecological Engineering

- **Habitat creation and restoration:** Creating and restoring habitats, such as wetlands, riparian zones, or buffer strips, is a key technique in ecological engineering. These habitats enhance biodiversity, provide nesting and foraging sites for wildlife, and act as buffers to minimize pollution runoff.
- **Biodiversity enhancement:** Promoting biodiversity is a fundamental aspect of ecological engineering. This can be achieved through techniques such as planting native vegetation, establishing wildlife corridors, or incorporating cover crops in agricultural systems. Increased biodiversity contributes to ecosystem resilience, stability, and the provision of multiple ecosystem services.
- **Biological control:** Utilizing natural enemies, such as predators, parasitoids, or pathogens, for pest control is an important component of ecological engineering. By enhancing the presence and activity of beneficial organisms, ecological engineering reduces reliance on chemical pesticides, minimizes ecological disruptions, and promotes natural pest control.
- **Agroforestry and permaculture:** Agroforestry and permaculture systems integrate trees, crops, and livestock, promoting ecological interactions and multiple functions. These systems enhance biodiversity, improve soil health, conserve water, and provide sustainable food production.

Benefits of Sustainable Management Strategies Using Ecological Engineering

- **Biodiversity conservation:** Ecological engineering contributes to biodiversity conservation by creating and enhancing habitats for native flora and fauna. Increased biodiversity supports ecosystem resilience, genetic diversity, and the conservation of endangered species.
- **Enhanced ecosystem services:** Sustainable management strategies utilizing ecological engineering enhance the provision of ecosystem services. Pollination, soil fertility, water purification, pest control, and climate regulation are improved through the promotion of biodiversity, functional diversity, and natural processes.
- **Climate change mitigation and adaptation:** Ecological engineering can

help mitigate and adapt to climate change impacts. Strategies such as reforestation, carbon sequestration, and the promotion of resilient agricultural systems enhance ecosystem capacity to sequester carbon, reduce greenhouse gas emissions, and mitigate climate-related risks.

» **Sustainable agriculture:** Ecological engineering offers sustainable solutions for agriculture. Techniques such as agroforestry, biological control, and cover cropping promote soil health, reduce chemical inputs, improve water management, and enhance crop productivity and resilience.

Examples of Sustainable Management Strategies

» **Constructed wetlands for water treatment:** Constructed wetlands utilize natural processes and diverse plant species to treat wastewater and improve water quality. These systems provide a cost-effective and sustainable approach to water treatment, while also enhancing wildlife habitat and biodiversity.

» **Riparian buffer strips for nutrient management:** Riparian buffer strips, consisting of vegetation along water bodies, can effectively reduce nutrient runoff from agricultural fields. These buffer strips act as filters, intercepting and absorbing nutrients before they enter waterways, reducing the risk of water pollution and promoting water quality.

» **Integrated pest management (IPM) in agriculture:** IPM integrates various ecological engineering techniques, such as biological control, habitat manipulation, and cultural practices, to manage pests sustainably. By combining multiple tactics, IPM reduces pesticide use, preserves beneficial organisms, and promotes natural pest control.

Integration of Ecological Engineering with Conventional Disease Management Approaches

The integration of ecological engineering with conventional approaches can lead to synergistic effects in disease management, reduced reliance on chemical inputs, and enhanced ecological sustainability. However, there are some challenges to integration, such as the need for knowledge and expertise, site-specific considerations, and compatibility with existing practices.

Some practical examples of integration include conservation biological control, habitat manipulation, crop rotation, and integrated pest management (IPM). Overall, the integration of ecological engineering with conventional disease management approaches offers a promising way to improve the sustainability and effectiveness of disease management.

Here are some specific examples of how ecological engineering can be used to complement conventional disease management:

- **Conservation biological control:** This approach involves creating habitats and providing resources to support populations of natural enemies of pests and pathogens. This can help to reduce the reliance on chemical control, as beneficial insects can help to control both pests and diseases.
- **Habitat manipulation:** This can be done by planting flowering plants, establishing hedgerows, or incorporating cover crops. These habitat modifications can enhance biodiversity, attract natural enemies, and promote biological control of diseases and pests.
- **Crop rotation:** This involves alternating crops with varying susceptibilities to diseases. This can help to disrupt disease cycles and reduce pathogen populations.
- **Integrated pest management (IPM):** This is an approach that integrates multiple disease management tactics, including ecological engineering techniques, chemical control, host resistance, and cultural practices. By combining multiple tactics, IPM can promote sustainable disease management, minimize pesticide use, and maximize the efficacy of ecological engineering strategies

Economic and Environmental Benefits of Ecological Engineering in Plant Disease Management

Ecological engineering is a field that applies ecological principles to design and manage ecosystems for sustainable outcomes. It can be used to manage plant diseases in a way that is both economically beneficial and environmentally friendly.

There are a number of economic benefits to using ecological engineering for plant disease management. These include:

- **Reduced chemical inputs:** Ecological engineering techniques can help to reduce the need for chemical pesticides and fungicides. This can save growers money on production costs.
- **Increased crop yield and quality:** Ecological engineering can help to improve plant health, which can lead to increased crop yield and quality. This can also lead to increased profits for growers.
- **Market opportunities and consumer demand:** Consumers are increasingly concerned about the environmental and health impacts of chemical inputs in food production. Ecological engineering practices, which minimize chemical

use and promote sustainability, can create market opportunities and meet the growing demand for environmentally friendly and socially responsible products (Tilman *et al.,* 2002).

There are also a number of environmental benefits to using ecological engineering for plant disease management. These include:

» **Reduced environmental pollution:** Ecological engineering techniques can help to reduce the release of synthetic chemicals into the environment. This can protect water resources, beneficial organisms, and overall ecosystem health.

» **Enhanced ecosystem services:** Ecological engineering can help to enhance ecosystem services, such as pollination, soil fertility, and natural pest control. This can contribute to sustainable agricultural systems and the preservation of natural resources.

» **Improved soil health:** Ecological engineering can help to improve soil health. Healthy soils support beneficial microorganisms, improve nutrient cycling, and enhance plant resilience to diseases. This can lead to long-term sustainability of agricultural systems.

» **Biodiversity conservation:** Ecological engineering can help to conserve biodiversity within agricultural landscapes. This can contribute to ecosystem resilience, genetic diversity, and the overall health of agroecosystems.

Some practical examples of the economic and environmental benefits of ecological engineering for plant disease management include:

» **Organic farming:** Organic farming relies on ecological engineering principles and offers numerous economic and environmental benefits. Reduced chemical inputs, enhanced soil health, and improved biodiversity contribute to lower production costs, increased market opportunities, and improved environmental sustainability.

» **Conservation biological control:** Conservation biological control is a key component of ecological engineering and promotes the activity of natural enemies for pest and disease control. By reducing reliance on chemical pesticides, growers can minimize production costs and protect beneficial organisms, leading to economic and environmental benefits.

» **Agroforestry systems:** Agroforestry systems, which combine trees with agricultural crops, offer economic and environmental advantages. These systems provide additional income streams from tree products, such as fruits or timber, while enhancing biodiversity, improving soil health, and promoting

ecosystem services, contributing to sustainability and profitability.

» **Integrated pest management (IPM):** IPM integrates ecological engineering techniques with other disease management approaches, emphasizing reduced chemical inputs and reliance on natural control mechanisms (Lopes *et al.,* 2019). By adopting IPM, growers can achieve economic savings, improved crop yield and quality, and reduced environmental impacts (Rodríguez-Rajo *et al.,* 2005).

Future Prospects and Challenges in Ecological Engineering for Plant Disease Control

Ecological engineering is a promising approach to plant disease control that can provide sustainable and environmentally friendly solutions. There are a number of emerging trends and technological advancements that have the potential to enhance the application of ecological engineering, such as precision agriculture, molecular tools, and climate-smart agriculture (Boller & Felix, 2009). However, there are also some knowledge gaps and socio-economic factors that need to be addressed before ecological engineering can be widely adopted.

One of the key challenges to the widespread adoption of ecological engineering is the need for a better understanding of ecological interactions and mechanisms underlying disease suppression. Research is needed to elucidate the roles of different organisms, such as beneficial microbes, predators, and parasitoids, in disease control. Additionally, studying the impacts of landscape structure, habitat connectivity, and plant-microbe interactions can enhance our understanding of disease dynamics in agroecosystems.

Another challenge to the adoption of ecological engineering is the need to scale up these strategies for large-scale implementation. Research is needed to assess the scalability of ecological engineering techniques, considering factors such as landscape heterogeneity, economic viability, and compatibility with existing agricultural systems. Understanding the socio-economic and policy factors that influence adoption is also crucial for effective implementation.

Despite these challenges, ecological engineering has the potential to revolutionize plant disease control by providing sustainable and environmentally friendly solutions. By investing in research, knowledge exchange, policy support, and stakeholder engagement, ecological engineering can become an integral part of sustainable and effective plant disease management in the future.

Conclusion

Ecological engineering is a promising approach to plant disease management that can provide sustainable and environmentally friendly solutions. By leveraging natural processes and enhancing biodiversity, ecological engineering can help to create more resilient and sustainable agricultural systems. There are a number of emerging trends and technological advancements that have the potential to enhance the application of ecological engineering, such as precision agriculture, molecular tools, and climate-smart agriculture. However, there are also some knowledge gaps and socio-economic factors that need to be addressed before ecological engineering can be widely adopted.

One of the key challenges to the widespread adoption of ecological engineering is the need for a better understanding of ecological interactions and mechanisms underlying disease suppression. Research is needed to elucidate the roles of different organisms, such as beneficial microbes, predators, and parasitoids, in disease control. Additionally, studying the impacts of landscape structure, habitat connectivity, and plant-microbe interactions can enhance our understanding of disease dynamics in agroecosystems. Another challenge to the adoption of ecological engineering is the need to scale up these strategies for large-scale implementation. Research is needed to assess the scalability of ecological engineering techniques, considering factors such as landscape heterogeneity, economic viability, and compatibility with existing agricultural systems. Understanding the socio-economic and policy factors that influence adoption is also crucial for effective implementation.

Despite these challenges, ecological engineering has the potential to revolutionize plant disease control by providing sustainable and environmentally friendly solutions. By investing in research, knowledge exchange, policy support, and stakeholder engagement, ecological engineering can become an integral part of sustainable and effective plant disease management in the future.

In conclusion, ecological engineering is a promising approach to plant disease control with the potential to provide sustainable and environmentally friendly solutions. However, there are a number of challenges that need to be addressed before ecological engineering can be widely adopted. By investing in research, knowledge exchange, policy support, and stakeholder engagement, the future of ecological engineering in plant disease management looks bright.

References

Altieri, M. A., & Nicholls, C. I. (2013). Agroecology and the design of climate change-resilient farming systems. *Agroecology and Sustainable Food Systems,* **37**(7), 869-890.

Bélanger, R. R., Labrecque, M., & Desjardins, Y. (2003). Strategies for managing foliar diseases of greenhouse tomatoes without the use of chemical fungicides. *Crop Protection,* **22**(1), 13-19.

Boller, T., & Felix, G. (2009). A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology,* **60**, 379-406.

Cook, R. J., & Baker, K. F. (1983). The nature and practice of biological control of plant pathogens. *The American Phytopathological Society.*

Ferrandino, F. J., & Smart, C. D. (2017). Practical applications of induced resistance to plant diseases: An appraisal of effectiveness under field conditions. *Journal of the American Society for Horticultural Science,* **142**(4), 243-255.

Gurr, G. M., Wratten, S. D., & Altieri, M. A. (2004). Ecological engineering for pest management: Advances in habitat manipulation for arthropods. CABI Publishing.

Haddad, N. M., Tilman, D., Haarstad, J., Ritchie, M. E., & Knops, J. M. (2001). Contrasting effects of plant richness and composition on insect communities: A field experiment. *The American Naturalist,* **158**(1), 17-35.

Jørgensen, L. N., Pauly, L., & Lübeck, M. (2017). Integrated pest management of plant pathogens: Epidemiological concepts, tools and methods. *Integrated Pest Management Reviews,* **22**(1), 18-47.

Kessel, G. J., Frinking, H. D., & Dijkema, G. P. (2004). Ecosystem services in sustainable agriculture. *Journal of Sustainable Agriculture,* **24**(3), 5-28.

Lefèvre, F., & Fravel, D. R. (2020). Combination of biological control agents and cultural practices for sustainable management of soilborne plant diseases. *Journal of Phytopathology,* **168**(5), 255-266.

Liang, J., Bi, Y., He, H., Fang, W., & Huang, X. (2018). Integrated control of wheat diseases by using plant-growth-promoting rhizobacteria. *Crop Protection,* **107**, 67-75.

Lopes, L. D., Hau, B., & Santos, A. (2019). Ecological engineering in plant pathology: Practices and perspectives. *Journal of Plant Pathology,* **101**(4), 1009-1024.

Malvick, D. K. (2016). Ecology and management of soilborne cereal pathogens: Status and challenges. *In Soilborne Diseases of Cereals and Their Control* (pp. 1-32). Springer.

Mäntylä, E., Klemola, T., & Laaksonen, T. (2017). Birds help plants: A meta-analysis of top-down trophic cascades caused by avian predators. *Oecologia,* **183**(1), 1-14.

Matsui, K., & Cowling, E. B. (2004). Chemical induction of disease resistance in plants. *Journal of Chemical Ecology,* **30**(5), 1003-1023.

Müller, C., & Hilker, M. (2017). Plant-insect interactions in chemically complex landscapes. *Annual Review of Entomology,* **62**, 451-473.

Poveda, K., & Rausher, M. D. (2011). Genetic variation and the evolution of resistance and tolerance in natural enemies. In Resistance Evolution in Pest Systems (pp. 129-147). Academic Press.

Rodríguez-Rajo, F. J., Jato, V., & Aira, M. J. (2005). Economic evaluation of the effects of plant disease control on crops: A review. *Spanish Journal of Agricultural Research,* **3**(3), 295-303.

Steinberg, C., & Edel-Hermann, V. (2019). Ecological engineering for plant protection: Concepts, applications, and perspectives. In Ecological Engineering for Pest Management (pp. 19-38). Springer.

Tamm, L., Thürig, B., & Bruns, C. (2018). Ecological strategies in organic and integrated apple production systems and their effects on plant health. *Frontiers in Environmental Science,* **6**, 4.

Thomas, M. B., & Blanford, S. (2003). Thermal biology in insect-parasite interactions. *Trends in Ecology & Evolution,* **18**(7), 344-350.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature,* **418**(6898), 671-677.

Van Elsas, J. D., Chiurazzi, M., Mallon, C. A., Elhottová, D., Kristufek, V., & Salles, J. F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences,* **109**(4), 1159-1164.

Vidal, S., & Kikkert, J. R. (2010). Integrated approaches to control soilborne fungal pathogens and pests of greenhouse crops. *Phytopathology,* **100**(8), 901-912.

14 Techniques in Phytopathology

Alby John[1], Deepa R. Chandran[2] and Saru Sara Sam[3]

[1,2,3]Department of Plant Pathology, College of Agriculture, Vellayani, Kerala Agricultural, University, Thiruvananthapuram- 695522, Kerala

Abstract

The detection and diagnosis of infection in plants has changed immeasurably over the years. Initially studies were conducted based on visual observations including signs and symptoms. Later with the advancement of science and technology, numerous techniques were developed. Starting from the culturing of microbes, morphological studies with the aid of different microscopy, biochemical tests, serological assay, molecular techniques till omic technologies and artificial intelligence for disease detection are discussed. Omics have provided various approaches to unveil the different key components responsible for disease resistance. All these techniques play a crucial role in understanding the pathogen biology, its host interaction and developing suitable management measures. Different novel methods are emerging day by day which will ultimately benefit by attaining sustainable food production.

Keywords: Isolation, staining techniques, microscopy, biochemical test, immunology, PCR variants, molecular markers, nucleic acid hybridization, omics approach, biosensors, artificial intelligence

Introduction

According to FAO 2022, it is estimated that 40 percent of global crop production is lost annually due to pest and diseases which cost the global economy over 220 billion dollars. The threat is further enhanced by the unprecedented climatic conditions, hindering the way towards sustainable development. Phytopathology plays a crucial role in diagnosing and detecting the disease caused by various microbes through

diverse techniques from basic to advanced level, which will ultimately benefit the farming community by deriving effective management strategies. Earlier records of disease identification were based on symptomatology which lacked accuracy and a more scientific approach was required. Later, methods for isolation, culturing and preservation of microbes developed. In the next stage, application of serological and molecular techniques deepened our knowledge on host pathogen interaction. Currently, the application of cutting-edge technologies like artificial intelligence and biosensors has given rise to crop forecasting models, where the farmer will get up to date information on health status of the plant. All these techniques from basics to advanced level have a significant role in understanding the pathogen biology, its host interaction and developing suitable management measures.

Techniques in Plant Pathology

Techniques in phytopathology can be broadly classified as direct and indirect methods. Direct method is a destructive approach, where samples from the field are taken for various lab analysis. Whereas, indirect method detect disease in the field itself without uprooting the plant i.e., based on variation in the light energy reflected and the volatile compounds released during stress condition. Direct methods comprise three main categories: conventional, serological and molecular techniques (Burns, 2009).

1. Isolation, purification and inoculation techniques for plant pathogens

Fungal pathogens are isolated from infected tissues by surface sterilization and plating on potato dextrose agar (PDA) medium, soil borne pathogens through serial dilutionof soil samples and baiting techniques for certain types of organisms. Examples of different baits used for some pathogens are cotton twigs (*Rhizoctoniabataticola*), apple (*Pythium*and *Phytophthora*), carrot disc (*Theilaviopsis paradoxa)*, sorghum seeds (*Sclerotiumrolfsii*), paddy culms (*Dreschlera oryzae*) and sugarcane disc (*Ceratocystisparadoxa*).Fungal pathogens isolated through these techniques are then purified by single spore isolation and hyphal tip method. Single sporing involves the transfer of a single germinated conidium to obtain a pure culture. This method is suitable for fungi that produce spores in culture E.g., *Verticillium*, *Fusarium*, *Alternaria*, *Colletotrichum*, *Phoma* etc. Hyphal tip method involves the transfer of a single hyphal tip to obtain a pure culture which is commonly done for *Pythium*, *Phytophthora*, *Rhizoctonia*, *Sclerotium* etc.

Bacterial pathogens are isolated from infected tissues (leaf, stem) by surface sterilization and plating on nutrient agar (NA) medium, through serial dilution of soil samples and form bacterial suspension. Purification of bacteria is done through pour plate, spread plate, simple streak and quadrant streak technique.

Koch postulates are performed on host plants under controlled conditions to prove the pathogenicity of the pathogen isolated from infected sample. According to the characteristics of the pathogen, host and process of infection different inoculation techniques is done on root, seeds, leaves and inflorescence *i.e.,* natural exposure of inoculum or direct application to host parts through spray inoculation, leaf clipping, pin pricking, swabbing, root dipping, leaf injection, seed inoculation, soil inoculation etc. can be done(Darshan *et al*., 2021).

Unlike fungi and bacteria, viruses are obligate intracellular organisms which require living cells to support their replication. Purification is the process of separating virus particles from the host cell constituentsthrough extraction, clarification, concentration and final purification. Virus is extracted using citrate, phosphate or borate buffer depending on the virus to be purified. Generally,phosphate buffer of 0.1 M and pH 7.4 is used. Other components of the extraction medium like metal ions (Ca^{2+}, Mg^{2+}), reducing agents (2- mercaptoethanol), additives (bentonite clay), chelating agents (Na-EDTA), detergents (Triton X-100) etc. keep the virus intact without aggregation during the isolation procedure.The separation of virus particle from the host cell is called clarification, also referred as partial purification.Concentration of the virus is performed through ultra centrifugation, PEG precipitation and salt precipitation. Later final purification is done through repeated centrifugation steps and virus layer is manually collected using hypodermic syringe. If differentviral properties like amino acid composition, nucleotide composition, percentage of protein, sedimentation profile etc. do not change upon further purification, the preparation is said to be pure (Hull, 2002).

2. Staining methods

Table 1: Staining for bacteria (Jayaramanand Verma, 2002)

Staining method	Stain used	Description
Simple	Methylene blue /safranin / carbol fuschin / crystal violet	Helps to determine cell shape, size and arrangement of bacterial cells
Gram staining	Crystal violet (primary stain), iodine (mordant), alcohol (decolourising agent), safranin (counter stain)	Used to distinguish gram positive and negative bacteria. Bacteria which appear blue are gram positive, red are gram negative.

Acid fast staining	Carbol fuchsin (primary stain), alcohol (decolourising agent), methylene blue (counter stain)	Acid fast bacteria become red and non-acid fast turns blue
Endospore staining	Malachite green (primary stain), safranin (counter stain)	Cells turn pink and endospores light green
Capsular staining	Nigrosin, alcohol (decolourising agent), crystal violet (counter stain)	Thin light region around bacterial cell under 100 X
Flagella staining	Tannic acid and potassium alum (mordant), carbol fuschin	Flagellacan be observedunder 100 X

Table 2: Staining for fungi (Darshan *et al*., 2021)

Staining method	**Description**
Lactophenol cotton blue staining (LPCB)	Used for examining all types of fungi by staining fungal cytoplasm. Hyphal elements turn deep blue against a light blue background.
Giemsa staining	Components are methylene blue (turns cytoplasm blue), eosin (stains nucleus red), methanol (fixative agent). Used to distinguish between *Rhizoctonia solani* and*Rhizoctonia* like organism by counting number of nuclei.
Calcofluor white stain	Calcofluor white binds to cellulose and chitin and Evan's blue is the counter stain. Fungi appear bright green to blue and remaining turns reddish orange.
Tryptan blue	It is a diazo dye that stains chitin and used in confocal microscopy for imaging vesicular arbuscular mycorrhizae (VAM)

Staining for phytoplasma

Diene's stain and DAPI (4', 6 –diamidino – 2 – phenylindole) stain are used for visualizing phytoplasma of infected tissues.Diene's stain is a nonspecific stain that rapidly diagnoses field samples. Phloem of infected tissues will be stained dark blue, while ylem turquoise, cortex light blue and cellulose cell wall appears yellow. One major drawback is its low sensitivity.DAPI is quick and easy detection method that binds strongly to adenine-thymine rich regions in DNA, so that phytoplasma with low GC content can be visualized in a fluorescence microscope. Phloem cells of infected tissues show bright fluorescence which is absent in healthy tissues (Darshan *et al*., 2021).

3. Microscopy

Microscopic studies provide the means to study pathogen biology and host pathogen interaction studies.

Table 3: Different types of microscopes (Mann *et al*., 2010)

Microscope types	Description
Simple microscope	Consist of one set of lenses and have low magnification power. Requires sufficient light source.
Compound microscope	Magnification power of 1000- 2000 X. Objective lens provide the primary magnification which is added up by the ocular lens.
Stereo microscope	Provides 3 D view of the specimen and used for dissection purpose. Magnification is 50 – 100 X.
Bright field microscope	Used to examine stained specimens which forms dark image against a brighter background. Live cells cannot be observed and magnification is 1500 X.
Dark field microscope	Both live and unstained samples appear light against a dark background. Internal structure of microorganisms can be observed.
Phase-contrast microscope	Live organism is observed in its natural condition and no staining is required. Magnification is 400 – 1000 X.
Fluorescence microscope	Fluorescence light is used to visualize specimens.Xenon arc lamp, mercury lamp or lasers are used as light source. Fluorescent stains like DAPI and Hoechst stain are used to visualize biological molecules.
Cone focal scanning microscope	Laser beam is used to illuminate the specimen. Image is digitally enhanced to view on computer.Optical imaging technology is used to increase optical resolution and is used in cellular morphology studies.
Transmission electron microscopy (TEM)	Short beam of electrons and magnetic condenser are employed to produce the image. Electron beam transmitted by ultra-thin section of a specimen is focussed by lens system to form a 2 D image.

Scanning electron microscopy (SEM)	Electron beam is projected on the sample which rapidly moves and scans the surface of the specimen and backscatters secondary electron which produce a 3 D image to visualize surface structure of specimen.
Scanning transmission electron microscopy (STEM)	TEM with additional scanning coils, detectors and circuits to make it aligned to SEM.
Reflection electron microscopy (REM)	Combination of imaging, diffraction and spectroscopy techniques for characterization of topography and crystal structure. High energy electrons are incident at glancing angles to the surface and reflected electrons are used to form REM image.

4. Biochemical test for characterization of bacteria

Many species of bacteria are similar in size, shape and arrangement. Hence, bacterial identification is done mainly based on their biochemical activities.

Table 4: Different biochemical tests (Darshan *et al.*, 2021)

Biochemical test	Description
3 % KOH test	Differentiate gram positive and negative bacteria. Gram negative bacteria produce viscous water upon lifting the culture containing loop.
Catalase test	Differentiate aerobic and anaerobic bacteria. Aerobic bacteria produce effervescence with addition of 3% $H_2O_2$to the culture.
Levan production	To test the sucrose utilization. Levan sucrase on 5% sucrose agar medium produces translucent shining colonies with raised convex structure.
Vogues Proskauer test	Differentiate Enterobacteriaceae sub groups where organisms convert glucose to acetone and culture turns pink. E.g., *Erwinia*
Methyl red test	To check the acidity and fermentation of the culture. Methyl red indicator added to the samples which turns red is acidic. E.g., *Erwinia*
3-ketolactose test	Differentiate *Agrobacterium* spp. *A. tumefaciens*produces 3-ketolactose and forms a yellow ring of Cu_2O around the cell mass. *A.rhizogen* and *A. vitis* produce negative result.

H_2S test	Differentiate Enterobacteriaceae members by H_2S production. Lead acetate strip is suspended over culture inoculated on sulphide indole medium. Black colouration along the line of bacterial colony indicates H_2Sproduction. E.g., *Erwinia*
Indole production test	To test the ability to produce tryptophanase. Kovac's reagent is added to the culture and cherry red colour indicates positive result.
Gelatin hydrolysis test	To identify the gelatinase producing capacity of the organism. When $HgCl_2$is flooded to culture grown on gelatin agar medium, clear zone around the culture indicates gelatinases. E.g., *Bacillus*
Starch hydrolysis test	To test the starch utilization activity of bacteria by flooding Legol iodine solution on NA with 0.2% soluble starch. Colourless zone around bacterial culture against a dark blue background indicates amylase production. E.g., *Ralstonia*
Citrate utilization test	Citrate enzyme production is observed by color change of inoculated sodium citrate agar containing bromo thymol blueto green. E.g., *Xanthomonas*
Gas production test	Culture is inoculated to two test tubes containing Leifon's medium with 1 % D- Glucose and Durum tube. Gas production is noted by the bubble formation. E.g., *Bacillus*
Acid production test	Carbon medium is amended with bromocresol purple dye and a carbon source. Yellow colour indicates the acid production.
Siderophore production	Fe^{3+}utilization of bacteria is identified by amending Succinate medium with indicator Chromazurol S. Formation of yellow colour around bacterial growth in blue coloured medium indicates siderophore production. E.g., *Pseudomonas fluorescens*
Fluorescent production	Fluorescent pigment productionis detected by exposing culture grown onKing's B media towards UV light. E.g., *Pseudomonas*
Potato soft rot test	To distinguish pectolytic and non-pectolytic*Erwiniae.* Rotting indicates pectolytic activity and slight rot shows negative result. E.g., *Erwiniacarotovora*
Klement's hypersensitive test	To confirm the pathogenicity of bacteria by inoculation of test bacteria by injection filtration on tobacco leaves. Sudden necrosis of the tissue within 12-24 hrs on infiltrated point indicate positive response.

5. Immunology based methods

Serology deals with specificity of antigen-antibody reaction and is widely applied for detection, diagnosis and characterization of plant pathogens. Serology can be used to detect latent infection, to develop virus free propagating material, to assess pathogenicity quantitatively, to detect vector borne pathogen and to conclude a relationship between pathogen related isolates and strains.Modern serological tests include ELISA, Immunoblotting, SDS PAGE, western blotting, Immunofluorescence, ISEM (Burns, 2009).

i. Enzyme Linked Immunosorbent Assay (ELISA)

Quantitative serological technique in which antigen – antibody reaction is determined by enzyme measurement. Enzyme (alkaline phosphatase, horse radish peroxidase) converts colourless substrate to coloured ones indicating the presence of antigen-antibody binding. Antigen of interest is adsorbed on a polystyrene plate with 96 micro wells and the unwanted components are washed away after each step, leaving only the specific reactants which is finally treated with enzyme and quantified by ELISA reader or spectrophotometry. ELISA can be broadly classified as direct and indirect based upon the type of labelled antibody. Direct ELISA is more specific and primary antibody directly binds to the test antigen whereas indirect ELISA have secondary antibody bind to the primary antibody which is bound with the test antigen. It is less specific and is used for broad spectrum detection of viruses.

Different types of ELISA are Direct Antigen Coated (DAC – ELISA), Double -Antibody Sandwich (DAS – ELISA), Protein A Sandwich (PAS) ELISA and Immuno-precipitation ELISA (IP-ELISA).

Direct Antigen Coated (DAC – ELISA)

Antigen is immobilized to the surface of multi-well plate. Direct DAC-ELISA is done by immobilised antigen detected by an enzyme conjugated primary antibody specific to the antigen. Indirect DAC-ELISA involves primary antibody binding to the immobilised antigen and a labelled antibody binds to the primary antibody for detection.

Double -Antibody Sandwich (DAS – ELISA)

Two antibodies specific for different epitopes of the antigen is required. One of the antibody coated on the surface of polystyrene plateimmobilizes the antigenand a detection antibody later added binds to the adsorbed antigen leading to the formation of antigen-antibody complex.

Protein A Sandwich (PAS) ELISA

Protein A, which is obtained from the cell wall of *Staphylococcus aureus* binds with the Fc portion of IgG. Bottom of the ELISA plate is coated with protein A followed by addition of antigen and antibody. Since the Fc region from the antibodies has affinity to protein A, the added antibodies will link with protein A at the bottom of the wellskeeping the virus in a specific orientation so that it will be free to trap the virus particles.

Immuno-precipitation ELISA (IP-ELISA)

Equal volume of infected plant extract and antiserum were centrifuged and resulting pellet were resuspended in ELISA extraction buffer for conventional indirect ELISA. It is used for detection of virus from different families such as *Cowpea severe mosaic virus(CPSMV)* and *Squash mosaic virus (SQMV)*.

ii. Immunoblotting

It is the serological detection technique, where the antigen-antibody complex is immobilized on nitrocellulose membrane.Using an enzyme conjugate and specific substrate results are visualized by the appearance of coloured spots on the membrane. Different immunoblotting techniques are DIBA, TIBA and dip stick assay.

Dot blots Immunobinding Assay (DIBA)

DIBA is a variant of ELISA where polystyrene plate is replaced with nitrocellulose membrane. Antigen is prepared by grinding tissues in Tris- buffered saline, containing one or more additives and a drop of test sample is spotted on the membrane. Bovine Serum Albumin (BSA) is used as the blocking agent. Membrane is placed in diluted antigen specific crude antiserum solution at 37° C for half an hour. Antibody probed with alkaline phosphatase is trapped and substrate NBT (nitro blue tetrazolium) is added, that convert enzyme linked to the IgG into a coloured material.

Tissue blot immunoassay (TBIA)

Quick method where the cut tissues are directly pressed on the membrane for transferring the antigen and the remaining procedure are similar to DIBA.

Dip stick ELISA

Nitrocellulose dip stick surfaces coated with specific monoclonal antibody are dipped into the sample and the antigens are detected by enzyme labelled virus specific antibodies (Darshan *et al*., 2021).

i. Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS PAGE)

It was first introduced by Lamelli in 1970. PAGE is a rapid technique for separation and analysis of protein mixtures based on their molecular weight by application of electric current. Four winter wheat cultivars (Sepahan, Shirody, Chamran, and Marvdasht) that differ in their resistance to powdery mildew were examined for the pattern of expression of the pathogenesis-related (PR) proteins transcripts via SDS PAGE. SDSPAGE findings revealed 25 bands on gel with high polymorphism in practically all genotypes (Kakaei *et al*., 2010).

ii. Immunosorbent electron microscopy (ISEM)

Immunofluorescence techniques helps in visualization of the distribution of molecules in a sample. Antibodies which are chemically conjugated to fluorescent dyes can be used to detect antigen-antibody interactions cytologically with the help of a fluorescent microscope after treating the tissues with proper reagents.

ISEM technique was first used by Derrick in 1973 to detect TMV and PVY. It is a combination of electron microscopy and serology*i.e.* ,the specificity of serological assay added with the visualization capability of electron microscope that provides serological and morphological details of the virus. It involves selective trapping of virus coated particle on carbon coated grid and adding antibody on virus particle. Skaff and Carroll (1995) developed a procedure for purifying barley yellow streak mosaic virus in barley and wheat. Polyclonal antibodies were produced from the concentrated virus preparations and were used in ELISA and ISEM for detecting the viral disease in host plants (Darshan *et al*., 2021).

6. Molecular methods used for detection of plant pathogens

Polymerase Chain Reaction (PCR) and its modifications

PCR is an ingenious tool for molecular biology for identification of plant pathogens. It is an *in vitro* method of nucleic acid synthesis by which a particular segment of DNA can be specifically replicated and was invented by Kary Mullis in1987. The double stranded DNA of interest is denatured to separate into two individual strands, each strand is then allowed to hybridize with a primer (annealing). The primer template duplex is used for DNA synthesis with the enzyme DNA polymerase. These three steps denaturation, annealing and extension are repeated several cycles to generate multiple forms of target DNA. It is simple, sensitive and quick. DNA extraction and amplification can be performed in a single day. It has wider applications such as mutation screening, genotyping, molecular ecology, molecular epidemiology, bioinformatics, genomic cloning, gene expression studies, DNA finger printing, genetic mapping etc.

6. Based on the purpose several modifications are made in PCR.

Table 5: Different types of PCR (Powell and Loeffelholz, 2018)

PCR variants	Description
Immunocapture PCR	Used in concentrating virus particles from plants with low virus titre. It involves capturing of virus particles by antibodies followed by amplification of nucleic acids by RT-PCR.
Colony PCR	Used to screen bacterial or yeast clones by picking it from an agarose plate followed by adding into PCR master mix.
Bio PCR	Viable bacterial cells are enriched in solid or liquid media and the cell growth is directly used for PCR.
Hot start / Cold finish PCR	Prevents nonspecific product amplification by arresting DNA polymerase activity at ambient temperature.
Inverse PCR	Detect flanking sequences near genomic inserts by a series of DNA digestion and self ligation which forms known sequences at either end of unknown sequence.
Step down PCR	Minimise the nonspecific amplification by gradually lowering the annealing temperature as PCR cycle progresses. Higher temperature provide greater specificity for primer binding and lower temperature allows more efficient amplification from the initial pcr products.
Asymmetric	Used in sequencing and hybridization probing where one DNA strand in a double stranded DNA gets amplified.
In-situ PCR	Detect minute quantities at early stage using internal probe by tissue fixing and subsequent treatment with proteolytic digestion.
Mini primer PCR	PCR targets small primer binding regions and amplifies conserved DNA sequences such as 16SrRNA.
Allele specific PCR	Selective amplification of specific allele based on single nucleotide polymorphism between alleles.

Long PCR	Two types of polymerases *i.e.*, non proofreading and proofreading polymerase together used for amplifying long targets.
Ligation mediated PCR	Small DNA linkers are ligated to DNA of interest and multiple primers annealed to DNA linker for DNA sequencing and DNA foot printing.
Rapid amplification of cDNA ends (RACE)	To analyse differential mRNA splicing and to identify transcription start and end site, it is necessary to obtain ends of cDNA sequences using known incomplete cDNA sequences.
Reverse transcription PCR (RT-PCR)	To isolate, amplify and identify a known sequence from tissue RNA. RNA isolated is converted into cDNA followed by PCR amplification.
Nested PCR	Increases specificity of DNA amplification by reducing non-specific amplification of DNA. Two primers are used. With first set of primers the PCR products obtained have the intended target and other non-specifically amplified DNA fragments. In the second reaction, the next set of primer which is having binding site partially different from original primer but within target DNA fragment is used. Performed for detecting various phytoplasma diseases.
Multiplex PCR	Multiple targets are amplified in a single PCR using different sets of primer in a reaction mixture. Large number of pathogens can be screened in a single reaction.
Real – time PCR	Used to amplify and simultaneously quantify a target DNA molecule. Real time increase in amount of DNA can be viewed with help of fluorescent reporter molecules like SYBR green.

7. Isothermal DNA amplification techniques

PCR thermal cycler is required for amplification purpose in majority of genotyping techniques. This can be replaced with economical isothermal DNA amplification methods.

i. Rolling circle amplification (RCA)

DNA polymerase mediated isothermal enzymatic process in which single stranded DNA molecules are synthesized on a short circular ssDNA template by using a single DNA primer. RCA was first done for dsDNA *Papillomavirus* and ssDNA *Geminivirus* (Inoue *et al*., 2006).

ii. Recombinase polymerase amplification (RPA)

RPA is a reliable isothermal amplification technique which quickly amplify and identify nucleic acids by using the properties of recombinase and related proteins. Kumar *et al*., (2018) developed and standardized sensitive, rapid, easy, and cost-effective RPA assay for detecting *Citrus yellow mosaic virus*. Primers are commercialized by TwistDx company. Virus identification is performed by adding recombinase which aids in primer extension, strand displacing polymerase and protein like single-stranded binding protein (SSB) for stabilizing the displaced strands (Londono *et al*., 2016).

iii. Loop mediated isothermal amplification (LAMP)

LAMP is a novel, rapid, simple and specific technique that can amplify nucleic acids which facilitates diagnosis of plant pathogen. It involves DNA polymerase and a set of 4 primers which recognize 6 distinct sequences on target DNA and depends on strand displacement activity to produce 10^9 copies in less than an hour. (Notomi *et al*., 2000).Samples are amplified through two types of elongation reactions occurring at the loop regions: self-elongation of templates from the stem loop structure formed at the 3'-terminal and the binding and elongation of new primers to the loop region (Notomi *et al*., 2015).It is widely applied for detection of fungi (*Fusarium oxysporum* f. sp. *cubense*, Li *et al*., 2013), virus (*Banana streak virus*, Peng *et al*., 2012), bacteria (*Xanthomonas*, Hodgetts *et al*., 2015) and phytoplasma (root wilt of coconut, Nair *et al*., 2016).

iv. Nucleic acid sequence-based amplification (NASBA)

It is used for the direct amplification of RNA by PCR using a reverse transcriptase, RNase H and, T7 RNA polymerase. It is highly sensitive and the end products are antisense to target viral sequences. Real time NASBA has been performed to detect *Strawberry vein banding virus* (SVBV, Klerks *et al*., 2001) and *Apple stem pitting virus* (ASPV, Vaskova *et al*., 2004).

v. Strand displacement amplification (SDA)

It is an isothermal, *in vitro* DNA amplification technique based upon the ability of Hinc II to nick the unmodified strand of a hemiphosphorothioate form of its recognition site, and the ability of exonuclease deficient klenow (exo$^-$klenow) to extend the 3'end at the nick and displace the downstream DNA strand. SDA reactions

involves denaturing of target DNA sample in presence of primers and other reagents followed by addition of Hinc II and exo$^-$kle now and finally the sample is incubated at 37°C (Walker *et al*., 1992).

Table 6: Isothermal amplification techniques (Darshan *et al*., 2021)

Isothermal techniques	Target	Primers needed	Initial heating	Incubation temperature °C	Limit of detection (copies)	Amplification (min)
NASB	RNA	2	No	41	1	60-180
SDA	DNA	4	Yes	30-55	10	60-120
RPA	DNA	2	No	37-42	1	20-40
RCA	DNA/RNA	1	Yes	30-65	10	60-240
LAMP	DNA	4-6	Yes	60-65	5	60

8. Nucleic acid hybridization techniques

Nucleic acid hybridization is a fundamental tool in molecular biology which have the capacity of single stranded nucleic acid to form double stranded molecules by standard base pairing. It is used for identification of gene of interest using DNA probes, which is a short fragment of DNA or RNA of variable length that can be radioactively or fluorescently labelled. It can then be used in DNA or RNA samples to detect the presence of nucleotide substances that are complementary to the sequence in the probe. Hybridization techniques have wide range of applications such as gene expression studies, screening specific clone from cDNA, identifying the location of gene in a chromosome, determining the pathogens present in a sample etc.

Hybridization technique can be broadly classified into solid support hybridization (southern, western, northern and eastern blotting) and In-silica hybridization (FISH and microarrays)(Kumar, 2021).

Southern blotting

Southern blotting technique was invented by Edwin M southern in 1975. It is a method for detection of specific DNA sequence in samples by immobilizingtarget DNA fragments into a membrane that have been fractionated by gel electrophoresis. DNA fragments separated on agarose gel are denatured, transferred and immobilized onto a membrane. The membrane is then exposed to a hybridization probe which is a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined. The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity which is visualized by autoradiography or tagging the molecule with a fluorescent or chromogenic dye.

Northern blotting

Northern blotting is a variant of southern blotting in which the target nucleic acid is RNA instead of DNA. In this technique, the amount and size of RNA transcribed from genes and their abundance is estimated. Since most plant virusesare having RNA as genetic material, it can be used in plant virus detection. It is used to study RNA degradation, half-life and gene expression studies at mRNA level.

Western blotting

It is the common technique widely adopted for the detection and analysis of proteins. SDS PAGE is done prior to western blotting where denatured protein mixtures are separated and blotted onto nitrocellulose membrane called as protein blotting. Later immobilized proteins form complex with specific antibody and the bound antibody is detected through different detection methods.

Eastern blotting

It is an extension of biochemical methods of western blotting to analyze lipid, carbohydrate epitopes, phosphomoieties and post protein translational modifications (Darshan *et al*., 2021).

Fluorescence in-situ hybridization (FISH)

It utilizes the combined features of microscopy and hybridization of DNA probes and target gene from plant sample. FISH follows the same principle of southern blot analysis and the target nuclear DNA is in interphase or metaphase stage. Fluorescent probes are prepared followed by denaturation and hybridization of probe and target. Finally, the target gene is located either through flow cytometry system or confocal fluorescence microscope (Kumar, 2021).

Microarray

DNA microarray also known as gene chip, DNA chip, bio array or gene array is an arrangement of thousands of genes fixed on a solid support like glass, silicon chips for expression profiling which detects the presence or absence of labelled nucleic acids in a sample.It is derived from southern blotting by combining miniaturization, automation, fluorescent labelling and parallelism allowing large scale monitoring of gene expression simultaneously.DNA microarrays are commonly used formRNA profiling, DNA-protein interaction, epigenetic status of genome and sequence polymorphism studies(Kumar, 2021).

9. Molecular markers

Unique DNA sequences, associated with a particular gene or trait found at spe-

cific locations of the genome showing polymorphism (base deletion, insertion and substitution) between different individuals and is detected by certain molecular technology. There are hybridization-based markers and PCR based markers. It can be used to study phylogeny, evolution, genome mapping, cultivar identification, mapping of R genes and gene pyramiding.

Table 7: Different molecular markers (Darshan *et al*., 2021)

Characters	**RFLP**	**RAPD**	**AFLP**	**ISSR**	**SSR**	**SNP**
Gene action	Codominant	Dominant	Dominant	Dominant	Codominant	Codominant
Reproducibility	High	High	Intermediate	Medium-High	High	High
Polymorphism	Medium	Very High	High	High	High	High
Required DNA	High	Medium	Low	Low	Low	Low
Cost	High	Less	High	High	High	Variable
Sequencing	Yes	No	No	No	Yes	Yes
PCR requirement	No	Yes	Yes	Yes	Yes	Yes
Visualization	Radioactive	Agarose	Agarose	Agarose	Agarose	SNP, VISTA
Required DNA (ng)	10000	20	500-1000	50	50	50

10. Advanced phenotypic and genotypic techniques for detecting plant pathogenic bacteria

i. The Biolog system

Biolog is a phenotypic technology based on carbon source utilization that detect microbes based on characteristic pattern from discrete test reactions performed within a 96 well microplate which use redox chemistry to calorimetrically indicate respiration of live suspensions. When a chemical is oxidized, cells reduce a tetrazolium dye, forming a purple colour that can be quantified spectrophotometrically at 590 nm. The characteristic pattern is called metabolic finger print or community level physiological profile (CLPP).*Enterobacter cloacaean emerging plant-pathogenic bacterium affecting chili pepper seedlings was identified using Biolog assay (Garcia-Gonzalez et al., 2018).*

ii. CABIQ (Classification automatique bacteries identification quarantine)

Rapid technique for detection of phytopathogenic bacteria based on phenotypic and genotypic properties of bacteria.It combines traditional identification test and molecular methods together. Around 500 reference strain are present in the database with conventional phenotypic test and the Biotype 100 galleries. Biotype 100 is a product for establishment of nutritional profiles for gram negative bacteria which

contains 100 tubes for studying carbon metabolism (Darshan *et al*., 2021).

iii. FAME (Fatty acid methyl ester analysis)

FAME analysis utilizes extracted fatty acid methyl esters for bacterial identification based on gas chromatographic analysis.It is produced through trans-esterification, where a glyceride reacts with alcohol in presence of alcohol to form fatty acid esters.Every microbe has a unique FAME profile with different quality and quantity of fatty acid. The structure of fatty acid varies with 9 to 20 carbon atoms and have produced characteristic profile for bacteria, fungi and nematodes. The amount of fatty acid present in cytoplasmic membrane and outer membrane of gram-negative bacteria are used in taxonomic studies (Kumar, 2021).

iv. PFGE (Pulsed field gel electrophoresis)

Genotyping technique to detect, identify and classify genome of microbes and mammalian cells accurately. It involves separating large segments of DNA using alternate and cross field.Standard DNA gel electrophoresis generally resolves fragments up to ~50 kb in size, PFGE separates DNA molecules up to 10 Mb. Large DNA molecules move in a zig zag form through the gel matrix, and such electrophoretic trajectories are disturbed in a size-dependent manner by carefully oriented electrical pulses. It is used for typing of bacteria, epidemiological studies, gene mapping in microbesand development of large-insert cloning systems such as bacterial and yeast artificial chromosomes (Herschleb *et al*., 2007).

11. OMICS – Novel approach for plant disease detection

During the last decade tremendous progress has been witnessed in the field of omics technologies which effectively reduced the time for developing better quality and disease resistant food crop. The term 'omics refers to the holistic analysis of the biological system involving the acquisition of vast data sets. Major omic technologies like genomics, transcriptomics, proteomics, metabolomics and phenomics provide insights into the complex molecular mechanisms underlying the plant–pathogen interactions and aids the discovery of pathogen's functional components involved in the disease. This knowledge can help to unravel the mechanisms by which disease resistance is conferred (Zanardo *et al*. 2019). It is a system-based approach to unveil complex network of interactions between genes, proteins, and metabolites (Rani*et al*., 2021; Kumar *et al*., 2015).

Genomics

Genomics is the study of the genetic material that constitutes the structure, function and expression of all genes in an organism.It uses a combination of recombinant

DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyse the structure and function of genomes.By using next generation sequencing techniques, a large amount of data is created that helps in faster and cheaper sequencing to understand the genome arrangement, biology and evolutionary history of thousands of organisms(Goodwin *et al*., 2016).

Genomics can be further classified into structural and functional genomics. Structural genomics unravel the complete DNA sequence of an organism, whereas functional genomics uses this genome sequence to assess the functions of genes (Leister *et al*., 2005).RenSeq is a comparative genomics tool to detect nucleotide-binding leucine-rich repeats (NLR). It was used for identifcation and cloning of anti-*Phytophthora infestans* NLR gene'Rpi-amr3i' from a wild potato relative, *Solanumamericanum*. Transgenic potatoes with expression of Rpi-amr3i gene were found to be fully resistant to *Phytophthorainfestans* in greenhouse conditions (Witek *et al*., 2016).

Transcriptomics

The total set of RNA transcripts generated by a person's genome under specific circumstances or in a specific cell is known as their transcriptome. It is studied via high-throughput methods, includingmicroarray analysis.Comparison of whole transcriptomics data based on RNA-seq enables identification and understanding of differentially expressed transcript patterns, novel genes and splice variants in a cell (Gao *et al*., 2013; Loraine *et al*., 2013; Merican *et al*., 2019). Most of the plant viruses have RNA genome, which is difficult to study using conventional molecular biology approaches. Though, with the advent of transcriptomics techniques, such as RNA sequencing (RNA-seq) and Degradome-seq, the knowledge on plant–virus interaction has widened enormously (Zanardo *et al*., 2019).

Proteomics

The proteome is the total set of proteins present in a biological unit, in a specific cell or tissue at a particular developmental or cellular phase (Claudia *et al*., 2018).Proteomes present in an organism rely on number of factors, such as plant's developmental stage, response to stress conditions (biotic and abiotic), origin of tissue being examined, and the cellular compartments being studied (Renaut *et al*., 2006; Sergeant and Renaut, 2010). Hence, proteomics is essential for understanding the molecular mechanisms vital to plant growth and development. It involves identification of primary amino acid sequence, estimation of their relative amounts, their post-translocation modification state, and interaction with other proteins or molecules and characterization of protein functions and structures (Barbrer-Brygoo and Joyard, 2004) via different strategies

including functional and structural proteomics, and protein–protein interaction (PPI) analysis. Protein expression profile aids to unravel the functions of diverse proteins by examining the plant responses to external stimuli, such as disease and insect attack (Van Emon, 2016).

Metabolomics

Metabolomics is an evolving field that focuses on comprehensive measurement of all metabolites and low molecularweight molecules present within cells, tissues or organisms (Castro-Moretti *et al.*, 2020). Metabolome data is generated using two main techniques - NuclearMagnetic Resonance (NMR) and Mass Spectrometry (MS). Metabolites are the final products of gene expression, which govern the phenotype of a plant in a particular physiological condition at the biochemical level. Pathogen infected plants modify their metabolome in the infected tissue, i.e., local response, and this response can be further extended to other tissues of the plant triggering a systemic response. Thus, metabolite profile patterns can deliver a deep understanding of the physiological condition and biochemical processes in a plant (Fiehn *et al.*, 2000).

Phenomics

Phenome is the set of phenotypes that are produced by an individual over the course of growth and development and phenomics refers to the measurement and analysis of the phenomes. It permits the quantification of morphological traits that are difficult to track visually and non-destructively (Chang *et al.*,2019). Crop phenotype arises from the interaction between the genotype (G) and the environment (E).As the environment changes, the complete characterization of phenome is not possible (Houle *et al.*, 2010). Statistical analysis is applied to spot these phenotypic variations within a genotype. Novel high throughput phenotyping techniques, such as non-invasive imaging, image analysis, robotics, spectroscopy, and high-performance computing, have enabledaccurate and faster phenotypic analysis of crop plants at early developmental stages in the natural field conditions as well as in controlled laboratory environment.

Bioinformatics

Recent omics technologies have created a large amount of biological data; therefore, sophisticated computational analyses are required to draw useful conclusions. Bioinformatics involves sequence analysis including identification of genes in DNA sequences, identification of families of related sequences, development of models, alignment of similar sequences and phylogenetic trees generation for examining the evolutionary relationships and discovering all the genes and proteins from a known

sequence (Sidhu *et al*., 2020). Biological databases are the collection of biological data that contain information in an efficient, structured, searchable, updated periodically and cross-referenced manner. The webaccessible open-source (http://www.prgdb.org) database— 'PRGdb' (Osuna-Cruz *et al*., 2018), provides inclusive overview of plant resistance genes. Basic Local Alignment Search Tool (BLAST)is a bioinformatics software tool for performing sequence alignment and is the quick method for identifying specific sequences from large data sets and annotating novel sequences. DOME is used for management of metabolomic, proteomic and transcriptomic data. Metabolomic pathway databases can be accessed through database such as KEGG (http:// www.genome.ad.jp/kegg/), MetaCyc (http://metacyc.org/) etc.

12. Biosensors

It is a self-contained analytical device that incorporates a biologically active material in contact with an appropriate transducer for the purpose of detecting the concentration or activity of chemical species in any sample. It consists of three main parts i) a bioreceptor – which may be an enzyme or any binding molecule, ii) a transducer, that transforms the signal resulting from the interaction of the analyte with the bioreceptor into a measurable signal and iii) a signal processing system which converts the signal into a workable form (Kumar, 2021).

13. Artificial intelligence

Artificial Intelligence is one of the booming technologies which is ready to create a new revolution in the world by making intelligent machines. In this era of modern farming, inclusion of artificial intelligence mediated smart technologies for rapid detection, accurate diagnosis and real time management of plant diseases will be a promising tool for sustainable agriculture. Artificial intelligence can be defined as the simulation of human intelligence in machines that are programmed to think like humans and mimic their actions. Application of AI techniques for plant disease detection can be classified as machine learning and deep learning. Image processing is the prerequisite step for both methods (Sujawat and Chouhan, 2021). Selvaraj *et al*. (2019) created an AI-based banana disease and pest detection system using three deep convolutional neural networks; ResNet50, InceptionV2 and MobileNetV1. Dataset consists of five major diseases (Banana bunchy top, Black sigatoka, Xanthomonas wilt in pseudostem, Xanthomonas wilt in fruit) along with their respective healthy classes. Both ResNet50 and InceptionV2 models have almost similar performance in all the cases compared to MobileNetV1. Based on the study, they developed 'Tumaini' mobile application for global banana farmers.

Conclusion

Plant diseases are responsible for huge yield losses. In a world where total crop failure can quickly lead to human misery and starvation, accurate diagnostics play a key role in keeping plants free from pathogens. Various phytopathological techniques from basics to advanced level discussed in this chapter are applied for early detection, diagnosis and management of diseases.The development of omics science and technology have opened up numerous avenues for the detailed explanation of the molecular mechanisms, pathways, and processes involved in disease development and have helped a great deal in the development of methods for disease prevention, diagnosis, examination, and management, thus revolutionizing agriculture.

References

Barbrer-Brygoo H, Joyard J (2004) Introduction—focus on plant proteomics. Plant Physiol Biochem. 42:913-917.

Burns, R. (2009). Methods in molecular biology. Humana Press.

Castro-Moretti FR, Gentzel IN, Mackey D, Alonso AP (2020) Metabolomics as an emerging tool for the study of plant–pathogen interactions. Metabolites 10(2):52

Chang T-G, Chang S, Song Q-F, Perveen S, Zhu X-G (2019) Systems models, phenomics and genomics: three pillars for developing high-yielding photosynthetically efcient crops. In Silico Plants. https://doi.org/10.1093/insilicoplants/diy003.

Claudia M, Demis AK, Jana V, John H, Nicholas WW, Patrick AL, Rafaele F (2018) Genome, transcriptome and proteome: the rise of omics data and their integration in biomedical sciences. Brief Bioinform 19(2):286–302. https://doi.org/10.1093/bib/ bbw114.

Darshan, K., Amrurthalakshmi, M., & Reddy M.G. (2021). Phytopathological techniques basic to advances.

FAO [Food and Agriculture Organization]. 2022. The state of food security and nutrition in the world: repurposing food and agricultural policies to make healthy diets more affordable [0n-line]. Available: https://www.fao.org/documents/card/en/c/cc0639en [20 Oct.2022].

Fiehn O, Kopka J, Dörmann P, Altmann T, Trethewey RN, Willmitzer L (2000) Metabolite profling for plantfunctional genomics. Nat Biotechnol 18:1157–1161.

Gao Y, Xu H, Shen Y, Wang J (2013) Transcriptomic analysis of rice(Oryza sativa) endosperm using the RNA-Seq technique. Plant Mol Biol 81:363–378.

García-González, T., Sáenz-Hidalgo, H. K., Silva-Rojas, H. V., Morales-Nieto, C.,

Vancheva, T., Koebnik, R., & Ávila-Quezada, G. D. (2018). Enterobacter cloacae, an emerging plant-pathogenic bacterium affecting chili pepper seedlings. *The plant pathology journal*, *34*(1), 1.

Goodwin S, McPherson JD, McCombie WR (2016) Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet 17:333–351. https://doi.org/10.1038/nrg.2016.49.

Herschleb, J., Ananiev, G., & Schwartz, D. C. (2007). Pulsed-field gel electrophoresis. *Nature protocols*, *2*(3), 677-684.

Hodgetts, J., Hall, J., Karamura, G., Grant, M., Studholme, D. J., Boonham, N., ... & Smith, J. J. (2015). Rapid, specific, simple, in-field detection of Xanthomonas campestris pathovar musacearum by loop-mediated isothermal amplification. *Journal of Applied Microbiology*, *119*(6), 1651-1658.

Houle D, Govindaraju DR, Omholt S (2010) Phenomics: the next challenge. Nat Rev Genet 11:855–866.

Hull, R. (2002). Matthew's plant virology, 4th edn. Academic Press.

Inoue, J., Shigemori, Y., & Mikawa, T. (2006). Improvements of rolling circle amplification (RCA) efficiency and accuracy using Thermus thermophilus SSB mutant protein. Nucleic acids research, 34(9), e69-e69.

Jayaraman, J. and Verma, J. P. (2002). Fundamentals of plant bacteriology. Kalyani publishers.

Jeong, J. J., Ju, H. J., & Noh, J. (2014). A review of detection methods for the plant viruses. *Research in Plant Disease*, *20*(3), 173-181.

Kakaei, M., Kahrizi, D., & Mostafaie, A. (2010). Study on powdery mildew disease related proteins expression in winter wheat cultivars via SDS-PAGE. *Biharean Biologist*, *4*(2), 169-171.

Klerks, M. M., Leone, G., Lindner, J. L., Schoen, C. D., & Van den Heuvel, J. F. J. M. (2001). Rapid and sensitive detection of Apple stem pitting virus in apple trees through RNA amplification and probing with fluorescent molecular beacons. *Phytopathology*, *91*(11), 1085-1091.

Kumar A, Pathak RK, Gupta SM, Gaur VS, Pandey D (2015) Systems biology for smart crops and agricultural innovation: filling the gaps between genotype and phenotype for complex traits linked with robust agricultural productivity and sustainability. Omics J Integr Biol 19(10):581–60.

Kumar, P. (2021). Biophysics and molecular biology tools and techniques. Pathfinder publication.

Kumar, P. V., Sharma, S. K., Rishi, N., Ghosh, D. K., & Baranwal, V. K. (2018). An isothermal based recombinase polymerase amplification assay for rapid, sensitive and robust indexing of citrus yellow mosaic virus. *Acta Virol*, *62*(1), 104-108.

Leister D (2005) Plant functional genomics. Food Products Press, New York.

Li, B., Du, J., Lan, C., Liu, P., Weng, Q., & Chen, Q. (2013). Development of a loop-mediated isothermal amplification assay for rapid and sensitive detection of Fusarium oxysporum f. sp. cubense race 4. *European Journal of Plant Pathology*, *135*, 903-911.

Londoño, M. A., Harmon, C. L., & Polston, J. E. (2016). Evaluation of recombinase polymerase amplification for detection of begomoviruses by plant diagnostic clinics. *Virology journal*, *13*, 1-9.

Loraine AE, McCormick S, Estrada A, Patel K, Qin P (2013) RNA-seq of Arabidopsis pollen uncovers novel transcription and alternative splicing. Plant Physiol 162:1092–1109.

Mann, S. K., Kashyap, P. L. and Kang, S. S. (2010). Plant Pathology – A competitive vision. Kalyani publishers.

Merican AF, Mirsafan H, Ripen AM, Mohamad SB (2019) Studies of Body Systems. In: Ranganathan S, Gribskov M, Nakai K, Sch nbach C (eds) Encyclopedia of Bioinformatics and Computational Biology, vol. 3, pp 94–102.

Nair, S., Manimekalai, R., Ganga Raj, P., & Hegde, V. (2016). Loop mediated isothermal amplification (LAMP) assay for detection of coconut root wilt disease and arecanut yellow leaf disease phytoplasma. *World Journal of Microbiology and Biotechnology*, *32*, 1-7.

Notomi, T., Mori, Y., Tomita, N., & Kanda, H. (2015). Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. *Journal of microbiology*, *53*(1), 1-5.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids research*, *28*(12), e63-e63.

Osuna-Cruz CM, Paytuvi-Gallart A, Donato AD, Sundesha V, Andolfo G, Cigliano RA et al (2018) PRGdb 3.0: a comprehensive platform for prediction and analysis of plant disease resistance genes. Nucleic Acids Res 46:D1197–D1201. https://doi.org/10.1093/ nar/gkx1119.

Peng, J., Fan, Z., & Huang, J. (2012). Rapid detection of Banana streak virus by loop-mediated isothermal amplification assay in South China. *Journal of Phytopathology*, *160*(5), 248-250.

Powell, E. A., & Loeffelholz, M. (2018). PCR and its variations. In advanced techniques in diagnostic microbiology (pp.327-346). Springer, Cham.

Rani, M., Mangat, H. K., Pathak, R. K., & Yadav, I. S. (2021). Harnessing the potential of omics for prevention and management of the complex crop plant's diseases. *Journal of Proteins and Proteomics*, *12*(3), 227-245.

Renaut J, Hausman JF, Wisniewski ME (2006) Proteomics and low temperature studies: bridging the gap between gene expression and metabolism. Physiol Plant 126:97–109.

Selvaraj, M. G., Vergara, A., Ruiz, H., Safari, N., Elayabalan, S., Ocimati, W., and Blomme, G. (2019). AI-powered banana diseases and pest detection. Plant Methods. 15(1). https://doi.org/10.1186/s13007-019-0475-z.

Sergeant K, Renaut J (2010) Plant biotic stress and proteomics. Current Proteomics 7(4):275–297.

Sidhu KS, Bhangu SK, Pathak RK, Yadav IS, Chhuneja P (2020) Identifcation of natural lead compounds for leaf rust of Wheat: a molecular docking and simulation study. J Proteins Proteom 11(4):283–295.

Skaf, J. S., & Carroll, T. W. (1995). Purification of barley yellow streak mosaic virus and detection by DAS-ELISA and ISEM using polyclonal antibodies. *Plant disease*, *79*(10), 1003-1007.

Sujawat, G. S. (2021). Application of Artificial Intelligence in detection of diseases in plants: A Survey. *Turkish Journal of Computer and Mathematics Education (TURCOMAT)*, *12*(3), 3301-3305.

Van Emon JM (2016) The omics revolution in agricultural research. J Agric Food Chem 64:36–44. https://doi.org/10.1021/acs.jafc. 5b04515.

Vašková, D., Špak, J., Klerks, M. M., Schoen, C. D., Thompson, J. R., & Jelkmann, W. (2004). Real-time NASBA for detection of Strawberry vein banding virus. *European Journal of Plant Pathology*, *110*, 213-221.

Walker, G. T., Fraiser, M. S., Schram, J. L., Little, M. C., Nadeau, J. G., & Malinowski, D. P. (1992). Strand displacement amplification—an isothermal, in vitro DNA amplification technique. *Nucleic acids research*, *20*(7), 1691-1696.

Witek K, Jupe F, Witek AI, Baker D, Clark MD, Jones JDG (2016) Accelerated cloning of a potato late blight-resistance gene using RenSeq and SMRT sequencing. Nat Biotechnol 34:656–660.

Zanardo LG, de Souza GB, Alves MS (2019) Transcriptomics of plant– virus interactions: A review. Theor Exp Plant Physiol 31:103. https://doi.org/10.1007/s40626-019-00143-z.

15 Dissemination and Survival of the Plant Pathogens

Vikram Singh[1] and Ashwani Kumar[2]

[1]Ph.D. Research Scholar, Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar - 125004 (Haryana)
[2]Assistant Professor, Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar - 125004 (Haryana)

Abstract

Plant pathogens pose a serious danger to global food security and causes huge annual yield loss of crops around the world. The initial step in the infection chain or disease cycle of plant pathogens is the means of survival. Both the terms "primary infection" and "primary inoculum" refer to the initial infection that develops as a result of the pathogen's sources of survival in the crop (infected hosts serving as inoculum reservoirs, saprophytic survival outside the host, dormant spores and other structures in or on the host or outside the host). The spores or other pathogen structures act as sources of secondary inoculums and secondary infections, which spread the illness throughout the field, after the disease has started to harm the crop. The spread of plant pathogens is the second link in the infection cycle. Dispersal, dispersion or transmission of plant pathogens refers to the movement of spores or infectious materials functioning as inoculum from one host to another host at varying distances that causes the disease to spread. The spread of the pathogen or illness is crucial for the continuation of the pathogen's life cycle and its evolution as well as for the spread of plant diseases. For successful management of plant diseases, it is crucial to understand these techniques of pathogen survival and spread since it is possible to stop these processes and breaking the chain of infection.

Keywords: Plant Pathogens, survival, dissemination, primary infection, disease cycle

Introduction

The majority of plant pathogens have evolved effective strategies for surviving during the unfavourable season of the year in order to maintain the infection chain, so that its infection might be revived when the favourable season arrives. Plant pathogen's main sources of survival include living hosts that are infected, contaminated or infected planting organs, crop residues, resting structures and soil. It is possible for *Phythophthora infestans* to persist in living host tissue, such as seed tubers, cull piles and volunteer potatoes that remain the winter in the field, Shinners *et al.* (2003), on other solanaceous plants and in the soil (Kirk *et al.,* 2013). Primary inoculum (PI), which is present in the soil, produces primary infection of the crop from healthy seed whereas infected tubers convey the infection to the field. The PI may also travel vast distances or from nearby areas by wind. The fungus then spreads spores on leaves. When these spores touch healthy plant surfaces, they spread by wind and water and start new diseases. This infection is a secondary one.

The disease is brought on by the main infection and it is disseminated by the secondary infection. Mycelium and conidia from the rice blast disease overwintered on straw, seeds, and collateral hosts including *Eleusine coracana*, *E. indica*, *Panicum* sp., *Setaria* sp. and others, serving as the main source of inoculums (Bhandari *et al*., 2017). *Rhizoctonia solani* is a soil and seed borne pathogen that survives in tropical conditions by developing sclerotia and mycelia in contaminated soil or seeds. Infected plant debris from weed or rice hosts is the main vector of the disease in soil (Figure 1). Sclerotia carried by crop residue and soil serve as the main source of inoculum in temperate zones, and they can spread from one field to another through irrigation water (Senapati *et al*., 2022). In the rice growing season, the bacterium that causes bacterial blight (*Xanthomonas oryzae* pv *oryzae*) persists in the roots of the weed "*Leersia haxandra*" from there, reaching the rice nursery beds and spreading further in the channels by the irrigation water applied to the young plants. In addition, the disease could enter the rice nursery by infected seeds or field found infected straw (Mizukami and Wakimoto, 1969).

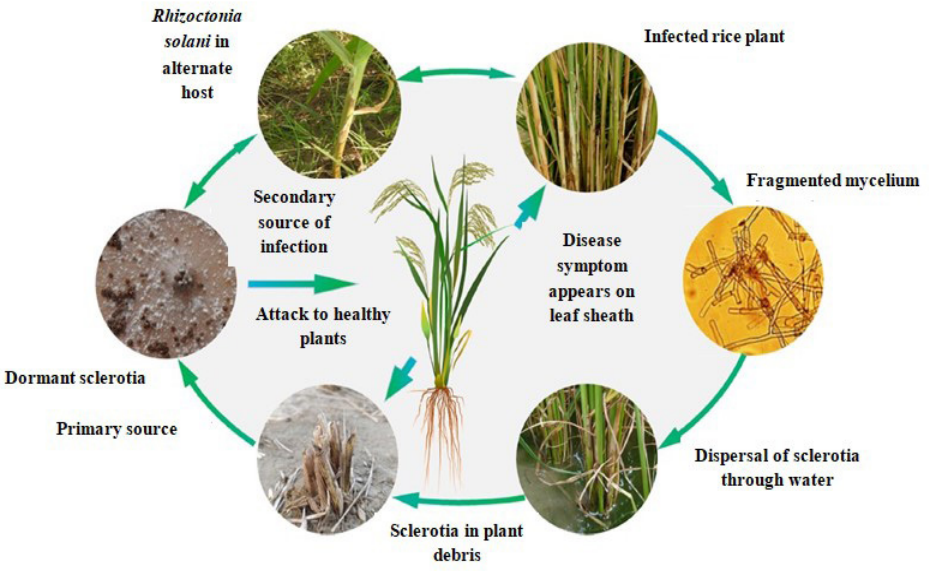


Fig. 1 Disease cycle of sheath blight of rice caused by *Rhizoctonia solani*

Senapati *et al*., 2022

After getting to the young rice seedlings, the pathogen begins to accumulate on the root surface and moves up towards the crown by using the metabolites, which typically ooze out from rice plant roots, for their proliferation (Mizukami, 1961). It is referred to as "dispersal" or "dissemination" when a plant pathogen spreads within the general region in which it is already established. When a fungus is actively growing vegetatively inside or on top of its host tissues, it produces both asexual and sexual spores, which are then mechanically distributed throughout time and space by a variety of methods. Infection of the mid-rib, lamina, leaf sheath, and stalk arises from the secondary transmission of the sugarcane red rot disease-causing fungus during the monsoon, which is mediated by irrigation, rain water, and rain splash. In the winter, air currents support the pathogen's spread (Sharma and Tamta, 2020). In bacterial diseases, the bacterial cells manifest as oozing on the host's surface or they may cause tissue breakdown, exposing the bacterial mass, which is then spread by a variety of physical and biological agents. Seeds, fungi, nematodes, insects and phanerogamic plant parasites are examples of external agents that might help transmit plant viruses (Van Regenmortel *et al*., 2000). Only a few plant viruses are transferred by humans via operational processes such vegetative plant multiplication, interculture activities, transportation of infected material from one location to another and introduction of new crops or varieties in current or new geographic areas. The majority of plant

viruses are spread by insect vectors. The two components of an animate pathogen's infection chain, namely the pathogen's ability to survive in latent structures and its ability to spread, are inextricably linked. Actually, the dormant structures offer a way of dispersion over time since they allow the disease to remain viable for an extended length of time and travel through physical mediums unharmed. This chapter describes many ways that plant infections can survive and spread, which will aid in understanding and control of plant diseases.

Survival of Plant Pathogens

The initial step in an infection chain or disease cycle is the means of survival. When a crop becomes infected for the first time, it is referred to as having a primary infection or primary inoculum. These sources of pathogen survival include infected hosts that serve as inoculum reservoirs, saprophytic organisms that survive outside of host, dormant spores, and other structures that can survive for long periods of time inside or outside of host.

Sources of Survival of Pathogens:

1) Infected host as reservoir of inoculum (or) survival in vital association with living plants.

2) Survival as saprophytes outside the host.

3) Survival by means of specialized resting structures in or on the host or outside the host.

4) Survival in association with insects, nematodes and fungi.

1) Infected host as reservoir of inoculum:

The infected host serving as reservoir of active inoculum is grouped into:

a) Seed: Plant diseases can infect seeds either externally or internally as they develop and mature into fruits or pods. A seed-borne disease is loose smut. The infected seed contains the fungus systemically. When contaminated seed is sown, it grows into a seedling and the fungus spreads throughout the body, colonising meristemic tissue. The mycelia mature when spikes form, resulting in smutted heads (Abrahim, 2019).

b) Collateral hosts / Alternative hosts (wild hosts of same families): Collateral hosts are plant species that are vulnerable to plant diseases that affect crop plants and can support the growth and reproduction of these pathogens off-season. In this way, the weed hosts facilitate the transition between two crop seasons. *Pyricularia grisea*, the fungus that causes rice blast disease, can thrive during the off-season of the rice crop and infect grass weeds like *Brachiaria mutica*, *Dinebra retroflexa*, *Leersia*

hexandra and *Panicum repens*. The conidia (inoculum) released from the weed host and spread by the wind as soon as a new rice crop is planted infect the new rice crop (Kumar *et al.*, 2021).

c) Alternate hosts (Wild hosts of other families): The function of collateral hosts is more significant than that of alternate hosts. But when a pathogen has a very broad host range (like *Sclerotium rolfsii, Rhizoctonia solani, Fusarium moniliforme* etc.) and is adaptable to a wide range of environmental conditions, the disease's alternate hosts become a crucial part of its survival. For the heteroecious rust pathogens to complete their life cycle, these alternative hosts are crucial. For instance, the barberry bush (*Berberis vulgaris*), which serves as the alternate host of *Puccinia graminis tritici* (the pathogen that causes black or stem rust on wheat), survives alongside the cultivated host in temperate zones (Kumar *et al.*, 2021). This wild host, which belongs to a separate family, is crucial to the fungus' survival in such conditions.

d) Self sown crops: Numerous plant pathogens accumulate in crops that are self-sown, planted voluntarily, and planted early. The pathogen (Rice tungro virus) and vector (*Nephottetix virescens*) are both present in self-sown rice plants.

2) Survival as saprophytes outside the host:

Many plant pathogens have the potential to live saprophytically, which allows them to persist in the absence of host plants. Typically, saprophytic life exists in or on the soil. Fungi can exist without the cultivated host plant as saprophytes and fall into one of three categories for study:

(i) Soil inhabitants: Organisms that persist permanently in the soil without their host plant called saprophytes. For instance, *Pythium, Rhizoctonia* and *Sclerotium* species.

(ii) Root inhabitants: These are more specialised parasites that coexist closely with their hosts in soil to live. As long as the host tissue in which they are residing as parasites has not entirely disintegrated, the active saprophytic phase continues. Examples include the *Fusarium, Verticillium* and cotton root rot (*Phymatotrichum omnivorum*) fungus that cause vascular wilt.

(iii) Rhizosphere colonizers: More resilient to soil hostility are the organisms that colonise the dead substrates in the root region and stay there for an extended period of time. For illustration, *Cladosporium fulvum*, a tomato leaf mould.

3) Survival as dormant spores or specialized resting structures:

Plant viruses have no resting stage and are transmitted through a continuous infection chain.

Phytopathogenic bacteria: Additionally, the plant bacteria do not create dormant

spores or other comparable structures. They continue to exist in either their active saprophytic stage on the remains of dead plants or their active parasitic stage in the current host.

Nematodes: Both the active parasitic phase on a living host and the dormant structures, such as eggs, cysts, and galls, generated in host tissues, allow them to persist (Jones *et al*., 2013). These structures might be found in seed lots or in the soil.

Phanerogamic parasites: Through seeds, they can endure in a dormant form for many years. *Orobanchae* seeds can remain in the soil for up to 7 years (Joel *et al*., 2007). Fungi are the only organisms among plant pathogens that create spores, which are similar to nematode eggs, and other resting structures for their inactive survival. The following categories can be applied to these dormant structures of survival.

1) Soil borne fungi:

a) Dormant spores: Conidia (Peach leaf curl pathogen, *Taphrina deformans*) (Akbar *et al*., 2023), Chlamydospores (Wilt pathogen, *Fusarium* sp.), oospores (Downy mildew fungi), perithecia (Apple scab pathogen, *Venturia inaequalis*) etc.

b) Other dormant structures include thickened hypha, sclerotia, microsclerotia and rhizomorphs (*Armillaria mellea*), as well as *sclerotinia sclerotiorum*, which causes cottony rot. Hyphae that are thickened Sclerotia Rhizomorphs Microsclerotia

c) Physical factors (such as high temperatures, radiation, desiccation and anaerobiosis), chemical factors (such as antibiotics and antagonistic chemicals produced by other microbes) and biotic factors (such as parasitism and predation by microflora and microfauna) are all factors that can affect a pathogen's ability to survive in soil.

2) Seed borne fungi:

a) Externally seed borne: spores that are dormant on the seed coat Ex: Covered smut of barley, grain smut of jowar, bunt of wheat etc (Martin *et al*., 2022).

b) Internally seed borne: Under the seed coat or in the embry, dormant mycelium using wheat as an example, loose smut (*Ustilago nuda tritici*) (Abrahim *et al*., 2019).

c) Factors affecting the survival of the pathogen on/in the seed are temperature and moisture.

3) Dormant fungal structures on dormant or active host

In grapevine downy mildew, apple powdery mildew, etc. The mycelium of the fungus may be present in a dormant form in the damaged twigs, or its oospores or perithecia may be entrenched in the tissues of the affected organs. In addition to using eggs, cysts, and larvae of plant parasitic nematodes as over seasoning structures, parasitic

phanerogams can also persist as seeds (Jones *et al*., 2013).

4) Survival in association with insects, nematodes and fungi

Numerous significant plant pathogens could live and overwinter inside an insect's body. According to Wielkopolan *et al*. (2021), the corn flea beetle is thought to be the primary vector for the acquisition and transmission of *Pantoea stewartii*. Nematodes or fungi found in the soil help plant viruses including wheat mosaic, tobacco necrosis, tobacco rattle, and tobacco ringspot viruses live in between crop seasons. The nematode *Xiphinema americana* is connected to tobacco ringspot. The viruses are carried internally by the fungus *Polymyxa graminis* (Wheat soil borne mosaic and Barley yellow mosaic) and *Spongospora subterranea* (Potato mop top virus) and are spread by the dormant spore (Campbell, 1996).

Dispersal of Plant Pathogens

A pathogen's primary and secondary inoculum must be spread from the source of survival to the vulnerable sections of a healthy plant in order for it to infect it. It is referred to as "dispersal" or "dissemination" when a plant pathogen spreads within the general region in which it is already established. Dispersal or spread of the inoculum can occur when it is moved a little distance or a great distance. However, the dissemination of pathogens is essential for the continuation of the life cycle and the evolution of the pathogen as well as the spread of diseases. The dispersal of infectious plant pathogens in space occurs through two ways:

1. Autonomous or direct or active dispersal.
2. Indirect or passive dispersal

1. Autonomous or direct or active dispersal

According to this strategy, plant infections spread through soil, seed, and planting materials during routine agronomic activities. Insects, wind, water, etc. play little to no significant influence in this sort of dissemination.

(i) Seed as the means of autonomous dispersal: The migration of pathogens and the transmission of diseases are significant since the bulk of farmed crops are developed from seed. Cuscuta seeds, ergot fungus sclerotia, smut, sori, and other dormant structures of the disease are found in seed lots and dispersed as seed contaminants. The bacterial cells or fungal spores that are found on the seed coat, such as in the smuts of barley, sorghum, etc., can travel great distances. The seed contains the latent mycelium of many fungus, which is widely dispersed. There are three different ways that seeds are dispersed: internally, externally, and through contaminated seed.

(a) Seed contamination: Seed-borne pathogens avoid direct contact with crop seeds that are still viable by passing through seed lots as separate contaminants. The seeds of the virus or parasite and the host are mixed together during crop harvest. Differentiating between pathogen seeds and host seeds can frequently be difficult. For example, rye ergot and pearl millet smut. Smut sori and ergots are easily mixed with the seed lots during harvest and threshing (Miedaner and Geiger, 2015).

(b) Externally seed borne: Close contact between the structure of the pathogen and seeds is established when it embeds itself on the seed coat as dormant spores or bacteria during crop growth or at the time of harvest and threshing. For instance, cotton with bacterial blight, short or loose sorghum, etc. Outwardly seed-borne organisms, such as the smut spores present in many diseases, have an inbuilt capacity for extended life and can survive for many years. Ex: According to Williams *et al.* (1971), the spores of *Tilletia caries*, also known as the stinking smut of wheat, and *Ustilago avenae*, also known as the smut of oats, are still viable after 18 and 13 years, respectively.

(c) Internally seed borne: The embryo may become infected by the pathogen while it is still developing in the ovary. They become internally seed-borne. A good example is loose wheat smut (Kumar *et al*., 2021).

Differentiate Seed infection and infestation

Seed infection: Only until the pathogen has made a connection with the tissues of the seed and has grown in or on it for some time can a seed become infected. For example, in loose smut of wheat, the fungus grows in the embryo's tissues and becomes dormant when the seed enters dormancy.

Seed infestation: The seed is contaminated and only acts as a carrier for the disease when the fungus or pathogen is present on the seed coat and in the seed lot.

(ii) The use of soil for autonomous dispersal: Parasites or facultative saprophytes that reside in the soil may do this. The pathogen may disperse through the soil, by developing there, or by moving soil contaminated with it. The former is referred to as dispersal in soil, whereas the later is referred to as dispersal by soil.

The three steps of dispersal in soil are as follows:

Dispersal in soil:

(a) Soil contamination: The pathogen infects the soil as it slowly travels from an infected region to a new one.

(b) Growth and spread of a pathogen in soil: Once a pathogen has entered the soil, depending on its ability for multiplication and dissemination, it may grow and spread.

The most important characteristics of the pathogen are its potential for saprophytic survival and its aptitude to adapt to the soil environment. A pathogen's ability to survive is influenced by a variety of characteristics, including high growth rates, rapid spore germination, increased enzymatic activity, the capacity to create antibiotics, and tolerance to antibiotics produced by other soil-microorganisms.

The three different types of pathogens in soil can be distinguished based on this competitive saprophytic activity. In the absence of host plants, specialised facultative parasites (Saprophytes) can survive in soil, but they are more dependent on the host plant's leftovers (e.g., *Armillariella mellea*, *Ophiobolous graminis* etc.). Facultative parasites without specialisation can live their entire lives in the soil (*Pythium* sp., and *Phytophthora* sp.) (Barton, 1957). The presence of an active host is necessary for soil-borne obligatory parasites like *Syncytium endobioticum* and *Plasmodiophora brassicae*. The diseases remain in the soil as dormant organisms such as oospores (*Pythium*, *Phytophthora*, *Sclerospora*, etc.), Chlamydospores (*Fusarium*), smut spores (*Ustilago*) (Barton, 1957) and sclerotia (*Rhizoctonia*, *Sclerotium*).

Dispersal by the soil

The pathogen is spread by the soil during agricultural operations using tools, irrigation water, employees' feet, etc. As a result, plant debris containing bacterial and fungal infections as well as fungus propagules spread over the field (Beaumont, 1954). Transporting soil and materials for disease proliferation from one place to another is the most important method of disease transmission. For instance, relocating papaya seedlings from a nursery where *Pythium ophiodermatid*, the pathogen in responsible for papaya stem or foot rot, is present, may spread the infection to newly excavated pits where the seedlings will be planted. Similar to this, fruit tree grafts that are shipped with dirt surrounding their roots may spread diseases present in the nursery to the orchards (Baker, 1959).

(iii) The plant and its organs as a method of self-sufficient dispersal:

Plants, plant components other than seeds used for vegetative growth, unprocessed field output, and plant debris that accumulates during cropping make up the third way of autonomous dissemination. For instance, Shinners *et al*. (2003) used seed tubers from the potato's original source to spread late blight. California was first exposed to citrus canker by Asian immigrants. California's environment was favourable for the outbreak there.

(II) Passive or indirect dispersal:

Plant pathogens can spread passively through both living and non-living entities.

(i) Insects: Insects either outwardly (epizoic) or inside (internally) transport plant diseases (endozoic). They have the ability to spread bacterial, fungal, viral, mycoplasmic, spiroplasmic, rickettsial, etc.

Fungal diseases:

Particularly significant in terms of external transmission are the fungi that generate conidia, oidia, and spermatia in honey secretions with seductive smells. Sorghum sugary disease for example. Fly, pollinating bees, and wasps that are used for both pollination and the transfer of plant pathogens are attracted to the ascomycetes' spermatial oozings at the entrance of the spermagonia. Internally, elm bark bugs are carriers of Dutch elm disease (*Ceratostomella ulmi*) (Wielkopolan *et al.*, 2021).

Bacterial diseases:

Flies (bees), ants, and leaf miners, respectively, disseminate the fire blight organism (*Erwinia amylovora*) and the citrus canker bacteria (*Xanthomonas axonopodis pv. citri*) (Anderson, 1924). The bacteria *Erwinia tracheiphila* that causes cucumber wilt (*Diabrotica undecimipunctata*) is spread by both the spotted cucumber beetle (*Acalymma vittata*) and the striped cucumber beetle (*Acalymma vittata*) (Wielkopolan *et al.*, 2021). The bacterium contaminates the mouthparts, moves to the beetle's gut, and spends the winter within the beetle while the beetles eat on the infected plant. Thus, the beetle helps the bacteria in two ways: by facilitating their survival and by facilitating their spread.

Viral diseases:

Insect vectors like aphids, whiteflies, leafhoppers, thrips, beetles, mealybugs and mites are responsible for transmitting the majority of plant viruses. Aphids are by far the most prevalent insect vector of plant viruses. Approximately 55% of the viruses that cause illness in plants are transmitted by the more than 200 species of aphids that have been identified as plant-virus vectors, as shown in Table 1. However, other vectors transmitted lesser amounts of plant viruses, these include leafhoppers (11%), beetles (11%), whiteflies (9%), most of these whitefly-transmitted viruses are begomoviruses (family Geminiviridae), although whiteflies are also vectors of criniviruses, ipomoviruses, torradoviruses, and some carlaviruses (Castillo *et al.*, 2011), as shown in Table 2, nematodes (7%), fungi and plasmodiophorids (5%), and thrips, mites, or mealybugs (2%) (Astier *et al.*, 2001).

Table 1: Aphid-transmitted plant viruses of different families and modes of transmission

S. No.	Family	Genus	No. of Spp.	Virus	Mode of Transmission
1	Bromoviridae	Alfamovirus	1	Alfalfa mosaic virus	Noncirculative
2	Bromoviridae	Cucumovirus	3	Cucumber mosaic virus	Nonpersistent
	Comoviridae	Fabavirus	4	Broad bean wilt virus-1	
	Potyviridae	Macluravirus	2	Maclura mosaic virus	
	Potyviridae	Potyvirus	91	Potato virus Y	
3	Caulimoviridae	Caulimovirus	9	Cauliflower mosaic virus	Noncirculative
4	Closteroviridae	Closterovirus	8	Beet yellows virus	Semipersistent
5	Sequiviridae	Sequivirus	2	Parsnip yellow fleck virus	
6	Sequiviridae	Waikavirus	3	Anthriscus yellows virus	
7	Luteoviridae	Enamovirus	1	Pea enation mosaic virus 1	Circulative
8	Luteoviridae	Luteovirus	2	Barley yellow dwarf virus	Nonpropagative
	Luteoviridae	Polerovirus	5	Potato leafroll virus	
	Luteoviridae	Umbravirus	7	Carrot mottle virus	
9	Rhabdoviridae	Cytorhabdovirus	8	Lettuce necrotic yellows virus	Circulative
10	Rhabdoviridae	Nucleorhabdovirus	7	Sonchus yellow net virus	Propagative

Ng *et al.*, 2004

Table 2: Whitefly transmitted plant viruses

S. No.	Virus family	Virus genus	Whitefly	Mode of transmission
1	Betaflexiviridae	Carlavirus	Bemisia tabaci	Nonpersistent/semipersistent
2	Closteroviridae	Crinivirus	Bemisia afer	Semipersistent
			B. tabaci	Semipersistent
			Trialeurodes abutilonea	Semipersistent
			Trialeurodes vaporariorum	Semipersistent
3	Geminiviridae	Begomovirus	*B. tabaci*	Persistent nonpropagative
			Trialeurodes ricini	Unknown

4	Potyviridae	Ipomovirus	*B. tabaci*	Semipersistent
5	Secoviridae	Torradovirus	B. tabaci	Undetermined

Source: Lapidot *et al.,* 2014

Mycoplasma diseases:

Plant MLOs are found in the phloem, and MLOs are transferred to other plants by insects that feed on phloem. Leaf hoppers are the main vector for the spread of mycoplasmal diseases. In the field, the Sesamum phyllody phytoplasma is reported to be transmitted by leaf hopper, *Orosius albicinctus* Distant (Prasad and Sahambi, 1982) and *Hishimonus phycitis* Distant (Nabi *et al*., 2015).

Nematodes:

Numerous nematodes operate as carriers of fungi, viruses and bacteria. Bacterial diseases: The ear cockle worm *Anguina tritici* disperses the bacteria *Corynebacterium tritici* or *Clavibacter tritici*, which causes wheat yellow ear rot. When these two pathogens co-occur, a complicated condition known as Tundu of wheat emerges. *Corynebacterium tritici* cannot spread or cause infection unless *Anguina tritici* carries it (Gupta and Swarup, 1972).

Fungal diseases: Nematodes also spread fungi that cause root rot and wilt, including *Phytophthora*, *Fusarium*, *Rhizoctonia*, *Verticillium*, etc., (Armstrong and Armstrong, 1948). Plant nematodes are essential in the transmission of some viral infections. The nematodes are known to spread a variety of soil-borne viruses. The Dorylaimoidea nematode genus *Xiphenema*, *Longidorous*, *Trichodorus* and *Paratrichodorus* are known to spread plant viruses. Nematode transmitted polyhedral viruses (NEPO) and nematode transmitted tubular (NETU) viruses are the two types of nematode transmitted viruses based on the shape of their particles (Brown *et al*., 1995).

NEPO viruses: Polyhedral virus particles that are transmitted by nematodes. They are typically spread by *Xiphenema* and *Longidorous* species. For instance, the viruses that cause tobacco ringspot, tomato ringspot, tomato black ring and arabis mosaic (Brown *et al*., 1995).

NETU viruses are tubular-particled nematode-transmitted viruses. *Trichodorus* and *Paratrichodorus* spread NETU viruses. For instance, tobacco rattling virus and pea early browning virus (*Trichodorus* sp). e.g., *Trichodorus pachydermis* (Brown *et al*., 1995).

(ii) Humans: When it comes to the transmission of plant pathogens, humans have a more direct than indirect impact. Some of the methods and tactics used by humans for spreading assistance are listed below.

Seed trade: Without taking the required precautions, importing and exporting

contaminated seeds causes diseases to spread from one country or continent to another. Diseases that are disseminated through seed and propagation materials or on them typically do so in this manner. Plant viruses have been accidentally transmitted by humans for as long as people have collected and carried seeds to new locations. It took a long time for this technique of distribution to be recognised. In 1699, Hellwig first noticed that outbreaks of ergot (*Claviceps purpurea*) on rye followed the sowing of ergot infected seeds (Baker and Smith, 1966).

Planting contaminated seed: The propagation of pathogens from field to field, orchard to orchard, locality to locality, or country to country is aided by the planting of contaminated bulbs, bulbils, corms, tubers, rhizomes, cuttings, etc. of vegetatively propagated plants like potato, sweet potato, cassava, sugarcane, banana, many ornamentals and fruit trees, etc. Humans involved in preparational cultivation, planting, watering, weeding, trimming, etc., spread plant diseases when utilising conventional farming methods. Spores and other external structures of fungi can be transported from plant to plant and from field to field on workers' clothing, shoes, hands and other materials (Berry and Davis, 1957).

Through the use of contaminated tools: When used in the field for various cultural operations (weeding, thinning, hoeing, etc.), contaminated instruments have the potential to transmit pathogens from one area to another. For instance, wilt and root rot are soil-borne illnesses. The movement of seeds from one plant to another is facilitated by the use of pruning and cutting knives on plants like banana with a bunched-up top for example.

By employing contaminated grafting and budding material: Grafting and budding between healthy and sick plants is the most effective method of spreading infections among horticulture crops.

(iii) Phanerogamic parasite dispersal: Dodders spread numerous economically significant plant viruses. Dodder establishes close biological contact through its haustoria, acting as a bridge between the diseased host plant and the healthy host plant. Plant virus transmission most likely includes the interaction of host and dodder cells. Tomato ring spot virus is transmitted by *Cuscuta gronovii* (Welliver, 1992) *Cuscuta* spp. *transmits* apple mosaic virus (Yarwood, 1955).

(iv) Birds: Certain fungi and parasite seeds that are flowering are dispersed by birds. Crows in the tropics devour the meaty, sticky and gelatinous berries of gaint mistletoe (*Dendrophthoe* sp.), then scatter the seeds on other trees with their waste. *Loranthus* seeds are dispersed by birds by their faeces and attachment to their beaks (Singh *et al*., 2016). While constructing their nests, birds transport pieces of dodder stem, enabling them to move to other areas. According to Scharf and Depalma (1981),

birds disperse the *Endothea parasitica* spores that cause chestnut blight.

(v) Domesticated and wild animals: Domesticated animals (cattle) die after ingesting live fungus propagules (spores, oospores or sclerotia) when eating diseased feed. This faeces serves as an inoculum source when utilised as manure and spread on the ground. Moreover, animals' legs and hooves can be used to transport soil-dwelling fungus, notably sclerotia.

Spread by some Non-living Entities

(i) Wind: The scientific word for the wind-borne transmission of diseases is anemochory. Wind transmission is influenced by the upstream air currents, wind speed, and the downward wind movements. Wind is an efficient carrier of viral, bacterial, and fungal propagules.

Fungi: It is common for fungi to produce diseases that are lightweight and easily spread by wind. A high number of spores and conidia are produced by fungal diseases, which have developed to release their spores with sufficient force and to produce spores that are incredibly thin and light to travel long distances. As an illustration, consider rusts, smuts, downy and powdery mildew. Both short- and long-distance distribution can be done with the help of the wind. Basidiospores from rust fungi, conidia from powdery mildew, and sporangia from downy mildew fungi are some of the spores employed for short-distance dissemination. The wind, which transports uredospores from the source of survival in the hills in the far north (Himalayas) and south (Nilgiris), is the only factor responsible for the annual recurrence of cereal rusts in the plains of northern India.

Conidia of *Alternaria, Helminthosporium* and *Pyricularia*, uredospores of rust fungi and chlamydospores of smut fungi are a few examples of long-distance dispersal-adapted spores (Anwar, 1949). Uredial rust fungi may travel considerable distances thanks to air currents, which makes it possible for destructive epidemics to spread across a wide area. For example, uredospores of *Puccinia graminis var. tritici* have been discovered up to 14000 feet above infected wheat fields. According to Atkinson (1953), reports of *Alternaria* spores at 8000 feet, *Puccinia recondita* spores at 12500 feet, and *Cronartium ribicola* spores at 14000 feet all contained similar information. In just two days, this fungus' uredospores can travel nearly a thousand miles across the United States, from Mexico in the south to Dakota and Minnesota in the north. Uredospores can travel up to 1100 miles in a wind of 30 miles per hour without losing viability if they are distributed at a height of 5000 feet.

It assists in the spread of nematode cysts and the seeds of phanerogamic parasites, together with fungus. The worm *Heterodera* major cysts that cause the molya disease

of wheat and barley are transported by dust storms from Rajasthan to Haryana. With the diseased material, certain pathogenic bacteria are carried over short distances by the wind. *Erwinia amylovora*, the bacterium that causes fire blight on apples and pears, exudes thin strands of exudate that can break off and be blown by the wind (Anderson, 1924). Although viruses and phytoplasmas are not directly transmitted by wind, the insect and mite vectors that carry them move in different directions and across greater distances depending on the direction and speed of the air.

(ii) Water: The term "hydrochory" refers to the spread of plant diseases by means of water. Viruses can germinate more quickly on a moist surface, therefore even while water is less crucial than air in the long-distance transfer of diseases, it is more efficient. The main routes through which water is distributed are surface running water and rain splash. Short-distance water surface flow during irrigation from canals and wells or following heavy rains is what moves the germs. The mycelial fragments, spores, or sclerotia of fungi like *Colletotrichum falcatum* (red rot of sugarcane), *Fusarium, Ganoderma, Macrophomina, Pythium, Phytophthora, Sclerotium*, etc. can be disseminated by rainwater or irrigation water. Long-distance spreading by water is only feasible when floods cover a larger area or when water flows farther from the sources of pathogen survival (ARK, 1932).

Rain splash dissemination is another term for splash dispersion. It is one of the most efficient means for the spread of bacterial plant diseases. The propagules may splash in small droplets and rest on neighbouring healthy surfaces that are susceptible to raindrops that strike sores, pustules, cankers, or even soil surfaces with force. Alternatively, the water droplets may be transported over considerable distances by the wind. Examples include bacterial leaf spot (*Xanthomonas campestris pv. oryzae*), bacterial leaf streak (*Xanthomonas campestris pv. oryzicola*), and green ear of bajra (*Sclerospora graminicola*). Bacterial and fungal spores that are already present in the air or on plant surfaces are conveyed downhill and deposited on healthy, sensitive plants when rain splashes or irrigation droplets fall from above. Water plays a critical role in the spread of plant diseases as well as the growth and spore release of numerous fungus. Additionally, it speeds up the processes of infection and spore germination.

Conclusion

Plant pathogens have a variety of strategies for surviving and spreading. They can benefit from a variety of biotic and abiotic variables in order to survive and grow to their full potential. Infection is a survival tactic for plant pathogens. The foundation for plant pathogens growth is primary infection. The source of pathogen survival in the crops is primary inoculum. Hosts that are infected serve as inoculum reservoirs. The spores or other pathogen structures act as sources of secondary inoculum and

secondary infection, which disseminate the disease across the field after the disease has started to build up in the crop. So, we conclude that plant pathogens are even smarter than our assumptions as they use different techniques in different conditions for their survival and dissemination. Pathogen populations can be reduced by the application of cultural control strategies, such as the proper management of crop residue, utilizing suitable harvesting and storage techniques. By reducing pathogen proliferation, dissemination and survival, this will aid in managing plant diseases.

References

Abrahim, A. (2019). Loose Smut of Wheat (*Ustilago tritici*) and Its Managements: A Review Article. *Journal of Biology, Agriculture and Healthcare*, *9*(8), 25-33.

Akbar, F., Khan, H., Khadim, N., Rahman, A. U., Ullah, R., Ahmad, N., Hadi, F., & Ke, C. (2023). Current status of peach leaf curl disease in Pakistan and future management strategies. *Agrobiological Records*, *12*, 22-33. https://doi.org/10.47278/journal.abr/2023.012

Anderson, P. J. (1924). Overwintering of tobacco wildfire bacteria in New England. *Phytopathology,14,* 132-139.

Anwar, A. A. (1949). Factors affecting the survival of *Helminthosporium sativum* and *Fusarium lini* in soil. *Phytopathology, 39*, 1005-1019.

ARK, P. A. (1932). The behavior of *B. amylovorus* in soil. *Phytopathology, 22*, 7.

Armstrong, G. M., & Armstrong, J. K. (1948). Non-susceptible hosts as carriers of wilt fusaria. *Phytopathology*, *38*, 808-826.

Astier, P., Autieroh, D., Baldisseri, A., Baldo-Ceolinm, M., Bannern, M., & Bassompierrea, G. (2001). Inclusive production of rho0 (770), f(0)(980) and f(2)(1270) mesons in muon-neutrino charged current interactions. *Nuclear Physics*, *B601*, 3-23.

Atkinson, R. G. (1953). Survival and pathogenicity of *Alternaria raphani* after five years in dried soil cultures. *Canadian Journal of Botany*, *31*, 542-547.

Bhandari, D. R., Khanal, M. P., Joshi, B. K., Acharya, P., & Ghimire, K. H. (2017). Rice Science and Technology in Nepal. *Government of Nepal. Crop Development Directorate (CDD) and Agronomy Society of Nepal, Kathmandu*.

Baker, K. (1959). Soil microbiology and root disease fungi. Epilogue. In C. S. Holton, Ed., *Plant pathology-problems and progress*, 1905-1958.

Baker, K.F., & Smith, S.H. (1966). Dynamics of seed transmission of plant pathogens. *Annual Review of Phytopathology, 4*, 311-334

Barton, R. (1957). Germination of oospores of *Pythium mamillatum* in response to exudates from living seedlings. *Nature*, *180*, 613-614.

Beaumont, A. (1954). Soil-borne diseases and crop rotation. *NAAS Quart. Rev*, *1953*, 108-111.

Berry, S. Z., & G. N. Davis. (1957). Formation of oospores by *Peronospora destructor* and their possible relation to epiphytology. *Plant Disease Reporter*, *41*, 3-6.

Brown, D. J. F., Robertson, W. M., & Trudgill, D. L. (1995). Transmission of viruses by plant nematodes. *Annual review of phytopathology*, *33*(1), 223-249.

Campbell, R. N. (1996) Fungal transmission of plant viruses. *Annual Review of Phytopathology*, *34*, 87-108. doi: 10.1146/annurev.phyto.34.1.87. PMID: 15012536.

Castillo, J.N., Olive, E.F., & Campos, S.S. (2011). Emerging virus diseases transmitted by Whiteflies. *Annual review of Phytopathology*, *49*, 2019_2248

Gupta, P., & Swarup, G. (1972). Ear cockle and yellow rot disease of wheat: nematode bacterial association. *Nematologica* , *18*, 320-324

Joel, D.M., Hershenhorn, J., Eizenberg, H., Aly, R., Ejeta, G., Rich, P.J., Ransom, J.K., Sauerborn, J., & Rubiales, D. (2007). Biology and management of weedy root parasites. *Horticultural reviews*, *33*, 267-349.

Jones, J.T., Haegeman, A., Danchin, E.G., Gaur, H.S., Helder, J., Jones, M.G., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M., & Perry, R.N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, *14*(9), 946-961

Kirk, W., Wharton, P., Hammerschmidt, R., Abu-el Samen, F., & Douches, D. (2013). Late Blight. MichiganState University Extension Bulletin E-2945. East Lansing, MI. Available on: http://www.potatodiseases.org/lateblight.html.

Kumar, S., Bhowmick, M. K., & Ray, P. (2021). Weeds as alternate and alternative hosts of crop pests. *Indian Journal of Weed Science, 53*(1), 14-29.

Lapidot, M., Legg, J.P., Wintermantel, W.M., Polston, J.E., 2014. Management of whitefly transmitted viruses in open-field production systems. *In advances in virus research*, *90*, 148-206.

Martín, I., Gálvez, L., Guasch, L., & Palmero, D. (2022). Fungal Pathogens and Seed Storage in the Dry State. *Plants*, *11*, 3167. https://doi.org/10.3390/plants11223167

Miedaner, T., & Geiger, H. H. (2015). Biology, genetics, and management of ergot

(Claviceps spp.) in rye, sorghum, and pearl millet. *Toxins*, *7*(3), 659-678.

Mizukami, T. (1961). Studies on the ecological properties of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, the causal organism of bacterial leaf blight of rice plant. *Bulletin* of the *Faculty* of *Agriculture*, *Saga University*, *13*, 1-85

Mizukami, T., & Wakimoto, S. (1969). Epidemiology and control of bacterial leaf blight of rice. *Annual Review of Phytopathology*, *7*, 51-72. https://doi.org/10.1146/annurev.py.07.090169.000411

Nabi, S., Madhupriya, Dubey, D., Rao, G. P., Baranwal, V. K., & Sharma, P. (2015). Characterization of phytoplasmas associated with sesame (*Sesamum indicum*) phyllody disease in North India utilizing multilocus genes and RFLP analysis. *Indian Phytopathology*, *68*(1), 112-119.

Ng, J.C., & Perry, K.L. (2014). Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology*, *5* (5), 505-511.

Prasad, S. M., & Sahambi, H. S. (1982). Sesamum phyllody - some new host records. *Indian Phytopathology*, *35*(1), 159-160.

Scharf, C. S., & DePalma, N. K. (1981). Birds and mammals as vectors of the chestnut blight fungus (Endothia parasitica). *Canadian Journal of Zoology*, *59*(9), 1647-1650.

Senapati, M., Tiwari, A., Sharma, N., Chandra, P., Bashyal, B. M., Ellur, R. K., & Krishnan, S. G. (2022). *Rhizoctonia solani* Kühn pathophysiology: Status and prospects of sheath blight disease management in rice. *Frontiers in Plant Science*, *13*, 881116.

Sharma, R., & Tamta, S. (2015). A Review on Red Rot: The "Cancer" of Sugarcane. *Journal of Plant Pathology and Microbiology*, *1*, 003.

Shinners, C. T., Bains, P., McLaren, D., & Thomson, J. (2003). Commercial potato production-disease management. *Guide to commercial potato production prairies. Western Potato Council. Available on: http://www. gov. mb. ca/agriculture//crops/potatoes/bda04s07*.

Singh, S.R., Phurailatpam, A.K., Lyngdoh, N. & Pandey, A.K. (2016). Loranthus legistrinus– A causal factor for khasi Mandarin (*Citrus reticulate* Blanco.) decline in Arunachal Pradesh. AJH. *11*(2), 368-372.

Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., & Lemon, S.M. (2000). Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. *Academic Press, San Diego*.

Welliver, R. A., & Halbrendt, J. M. (1992). Dodder transmission of tomato ringspot virus. *Plant Disease*, *76*(6), 642-642.

Wielkopolan, B., Jakubowska, M., & Obrępalska-Stęplowska, A. (2021). Beetles as plant pathogen vectors. *Frontiers in Plant Science*, *22*41.

Williams, S.T., Davies, F.L., Mayfield, C.I., & Khan, M.R. (1971). Studies on the ecology of actinomycetes in soil II. The pH requirements of *Streptomycetes* from two acid soils. *Soil Biology and Biochemistry*, *3*(3), 187-195.

Yarwood, C. (1955). Mechanical transmission of an apple mosaic virus. *Hilgardia, 23* (15), 613-628.

16 Host Plant Resistance

Gayathri V[1] and Nagajyothi G N[2]

[1]M.Sc, (Hort), Kittur Rani Channamma College of Horticulture (KRCCH), Arabhavi, University of Horticultural Sciences(UHS), Bagalkote, Karnataka-591218
[2]Ph.D. Scholar., College of Horticulture,Bangalore, University of Horticultural Sciences (UHS), Bagalkote, Karnataka-587104

Abstract

Organic plant production uses natural products and natural self -regulation processes occurring in ecosystem. One of the most effective and eco friendly approach in controlling the plant diseases is to adoption of biotechnological techniques in production of disease resistant plants. As it is sustainable with no negative impact on plant health and agronomy. The availaibility of innovative applications and molecular techniques opens up new useful possibilities to plant protection for sustainable and organic agriculture. The author presents the host plant resistance, its types, mechanisms, modern approaches for breeding disease resistance crops in plant pathology as a new plant protection strategy.

Keywords: Biotechnological approaches, Genetic engineering, Host plant resistance, Transgenic disease management.

Introduction

In India, population is growing faster day by day. As a result, huge demand in the nutritious, organic food supply for the mankind's balanced diet.

The plant diseases are the most threat factor which are causing significant yield losses in most of the agricultural and horticultural crops. Traditional plant breeding methods have been used to develop resistant cultivars to various diseases. It has become routine to transfer genes from one organism to another, genes conferring

disease resistance to crop plants have been introduced. However, this process is time consuming and limited availability of genetic resources for most of the crops. The most important reasons for limited genetic resources available for breeding are that many of the natural gene traits that may be beneficial in one plant tissue may be deleterious in other plant tissue and that are causing loss of genes pools recurring during the domestication and breeding of crop plant (Cook and Baker, 1983). According to Agrios (1988; 2005) biotechnology is the genetic manipulation and multiplication of any living organism through novel techniques and technologies such as tissue culture, genetic engineering and transgenic disease management resulting in the production of improved or new organism and products that can be used in variety of ways for the benefit of mankind. Modern technologies such as gene transfers could be accomplished by the gene or biolistic method and agrobacterium mediated method as direct method. Vector mediated method as indirect method (Agnihotri et al., 1989). This can facilitate new insights into the complex metabolite neighbourhoods that give rise to a given phenotype and may allow discovery of new target genes to modify a given pathway. Such genes can then be subject to new metabolic engineering efforts and applications. Biotechnology permits accurate diagnosis of plant diseases in present decades. ELISA and PCR techniques are used in the identification of viral and bacterial diseases. The aim to write up this review to bring up the modern biotechnological approaches in controlling of plant diseases.

Terminologies:

Disease resistance

The inherent ability of an organism to resist or withstand the pathogen/ its infection is called **Disease resistance.**

Host Plant Resistance (HPR)

'Those characters that enable a plant to avoid, tolerate or recover from attacks of insects under conditions that would cause greater injury to other plants of the same species'. - **Painter R.H. (1951)**

'Those heritable characteristics possessed by the plant which influence the ultimate degree of damage done by the insect'. - **Maxwell E.G. (1972)**

A physiological deviation from the normal functioning of the organism/ plant caused by pathogenic organisms is a disease and may be caused by fungi, bacteria or viruses.

Host – Pathogen relationship

A disease is the result of an interaction of genes governing resistance in the host with those of governing pathogenicity in the pathogen. The resistance of a crop to a physiological race of the pathogen depends not only on the genotype of the host for resistance but also on the genotype of the pathogen for virulence or aggressiveness.

H.H. Flor (1942) proposed the gene-for-gene hypothesis, according to which, 'for every gene for resistance in the host, there is a corresponding gene for pathogenicity in the pathogen'. It means that there are at least two alleles at a locus controlling resistance/susceptibility in the host (R-r) and two alleles at a corresponding locus in the pathogen (V-v) controlling virulence / aggressiveness leading to no disease / disease(Fig.1) (Agrimoon.com)

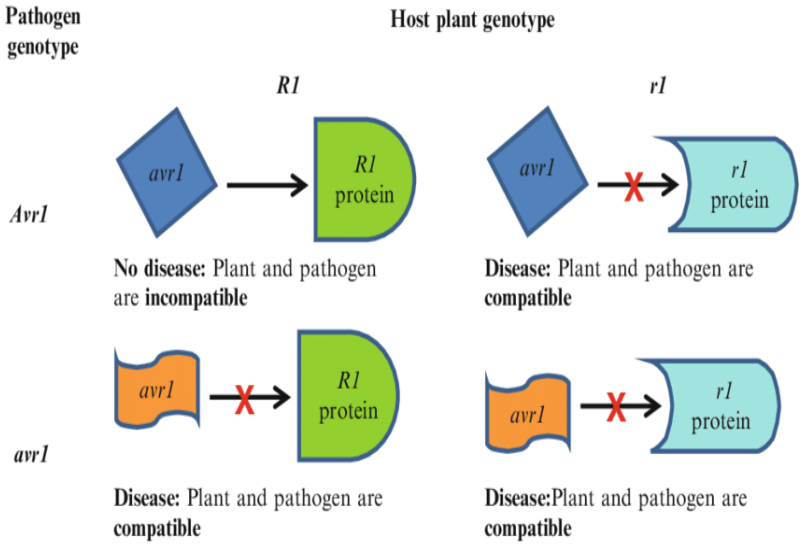


Fig.1 Gene-for-gene hypothesis (Agrios, 2005)

Types of Disease Resistance

1. **Ecological resistance or Pseudo resistance**

   Apparent resistance resulting from transitory characters in potentially susceptible host plants due to environmental conditions

   Pseudo resistance is classified into 3 categories

   a. **Host evasion**

      Host may pass through the most susceptible stage quickly or at a time when disease are less or evade injury by early maturing. This pertains whole population of the host

b. **Induced resistance**

Increase in resistance temporarily as a result of some changed conditions of plants or environment such as change in the amount of water or nutrient status of soil.

c. **Disease escape**

Absence of infection or injury to the host plant due to transitory process like incomplete infection. This pertains to few individuals of the host. (Agrimoon.com)

2. **Genetic Resistance**

A. **Based on the number of genes**

1. Monogenic resistance - controlled by single gene
2. Oligogenic resistance - controlled by few genes
3. Polygenic resistance - controlled by many genes
4. Minor gene resistance - controlled by many minor genes. Also called as adult resistance/mature resistance /field resistance /horizontal resistance
5. Major gene resistance - controlled by one or few major gene also called as vertical resistance

B. **Based on biotype reaction**

1. Vertical resistance – effective against specific biotypes (Specific resistance)
2. Horizontal resistance – effective against all the known bio types (Non-specific resistance)

C. **Based on population /line concept**

1. Pureline resistance – exhibited by lines which are phenotypically and genetically similar.
2. Multiline resistance – exhibited by lines which are phenotypically and genetically dissimilar.

D. **Multitrophic interactions**

1. Cross resistance – variety with resistance incorporated against a primary host to another host
2. Multiline resistance – resistance incorporated in a variety against different environmental stresses like insects, diseases, nematodes, heat, drought, cold etc

E. **Based on evolutionary concept**

1. Sympatric resistance – acquired by co-evolution of plant and insect (gene for gene) and governed by major genes.
2. Allopatric resistance – not acquired by co-evolution of plant and insect and governed by many genes.

Mechanism of disease resistance or Nature of disease resistance

Disease resistance is governed by several inbuilt mechanisms of the host plants against infection by the pathogen. They are disease escape, disease endurance or tolerance and true resistance. (Agrimoon.com)

a. Disease escape or Pseudo-resistance

It is a prevention mechanism that causes the host to escape pathogenic infection. Early or late maturity of the crop may prevent physical contact of the pathogen with the host. Mechanical and anatomical barriers such as thick cuticle, waxy bloom on leaves and stem, stomatal regulation prevent penetration of spores. **Ex:** Ergot (*Claviceps purpurea),* a fungal disease of inflorescence in cereals does not affect varieties of wheat and barley in which the flowers remain closed until pollination occurs. Erect leaves of barley avoid deposition of spores of *Erysiphe graminis tritici* in contrast to prostrate leaves. Early maturing varieties of groundnut escape early leaf spot infection (*Cercospora arachidicola*) and early varieties of wheat escape rust and loose smut infection.

A change in planting season has also been successfully employed as a measure of securing escape, e.g., the leaf rust of sugarcane (*Puccinia sacchari*) in the canal areas of Bombay severely affects cane when planted in June, but is of minor importance or absent in crops sown in October.

b. Disease endurance

The host after being infected by the pathogen tolerates the infection and suffers less damage. It does not result in any substantial decrease in yield. This is brought about by influence of external factors.

The plants fertilized with phosphatic and potash manures are more tolerant to disease in wheat against rust infection. Rice crops fertilized by silicates are resistant to blast (*Pyriculiaria oryzae*) in Japan. The fertilizers act indirectly to arrest vegetative growth and promote early maturity, better straw and strengthening tissues to protect the plant which form a bulwark against pathogenic invasion.

c. True resistance

It is the ability of the host plant to resist or withstand the attack of a pathogen. True resistance is inheritable and much less subject to environmental influence. It is specific in character. The basis of resistance may be morphological, functional, structural or protoplasmic. Functional nature of resistance is determined by opening of the stomata, time of opening of flowers and time of maturity, rate of cork formation and cambial activity.

True resistance is classified into 2 types:

Vander Plank (1960) has discussed the whole issue of disease resistance in a different perspective.

1. Vertical resistance (VR)

If more resistance to some races of a pathogen than to other races is called vertical resistance. It is also called perpendicular resistance, physiological resistance, seedling resistance, hypersensitivity, race specific resistance or qualitative resistance, unstable, often complete type of resistance. As it is conditioned by one or a few genes, it is called major gene or monogenic of oligogenic resistance.

2. Horizontal resistance (HR)

Resistance to more than one race of the pathogen or to many or all races of the pathogen is called horizontal resistance. It is non-specific resistance governed by polygenes. It is severally termed as non-specific, general, polygenic, minor gene, mature plant, adult, quantitative resistance, partial or field resistance or tolerance. HR causes reduction in the number and rate of sporulation of the pathogen on the host and slows down the infection rate.

Defense mechanisms of plants with its dominant plant genes against pathogens

1. Detoxification of pathotoxins

Pathogens that produce pathogenesis-related phytotoxins usually also have the capacity to metabolize i.e. detoxify, these compounds. The search for genes encoding the enzyme(s) performing the key catabolic step(s) should thus lead to a convenient source of resistance, which can be engineered into plants to protect them from the effects of the toxin. A gene encoding a tabtoxin acetyltransferase from the pathogen, *Pseudomonas syringae* pv. *tabaci* (wild fire disease of tobacco) was isolated and transferred into tobacco under a strong constitutive promotor. The transgenic plants expressed this gene and when treated with either the pathogen or its toxin, did not produce the chlorotic lesions typical of wild fire disease. (Agrimoon.com)

2. Activation of plant defense mechanism-Phytoalexins

Phytoalexins have long been known to accumulate in certain plants upon infection by pathogens. The production of phytoalexins is also triggered by mechanical stimulation, ultraviolet (UV) irradiation, stress and a variety of chemical elicitors. Phytoalexins are part of the localized hypersensitive response at the site of damage or pathogen ingress, which involves cell trauma and death. The importance of phytoalexins in the defense response is underscored by experiments and pathogenicity in *Nectria haematococca* was correlated to its ability to detoxify the phytoalexin, pisatin, by way

of demethylation. By transferring the demethylase gene from Nectria, *Aspergillus nidulans*, a non-pathogen on peas, was rendered insensitive to pisatin.

3. Defense related genes in plants

a. Single gene defense mechanism

There are some defense proteins which do not require any intermediate step both for their synthesis and their expression require only few steps and those genes encoding such proteins are called single gene defense mechanism. Chitinases and glucanases are those proteins belonging to single gene defense mechanism.

1. Chitinases and glucanases

Chitinases are abundant proteins found in wide variety of plants. There is strong correlative evidence that they are defense proteins with antifungal activity. Chitin is a major structural component of cell walls of many fungi. The low constitutive activity of chitinase found in many plants can be induced by wounding or by infection of the tissue with fungal pathogens. Chitinase with ß-1,3-glucanase (capable of degrading glucans present in fungal cell wall) degrades fungal cell walls and inhibits fungal growth at hyphal tips hyphal walls in plants. The chitinase and glucanase enzymes are having direct action against several fungal pathogens compared to other defense related proteins. Bean vacuolar chitinase gene under the control of the strong constitutive gene under the control of the strong constitutive promoter of the cauliflower mosaic virus (CaMV) 35 S transcript in tobacco and *Brassica napus*, which resulted in decreased symptom development by *Rhizoctonia solani*, the causative agent of post-emergence damping off.

An endochitinase gene (from genomic tomato DNA library) was introduced into *Brassica napus*. var. *oleifera*. The transgenic *Brassica* showed enhanced resistance against several fungal pathogens like *Cylindrosporium concentricum*, *Phoma lingam* and *Sclerotinia sclerotiorum* under field conditions when compared to non-transgenic plants. More recently, chitinase gene from *Manduca sexta*, tobacco horn worm, has been cloned into *P. fluorescens* to increase their antagonistic potential against *R. solani*. (Agrimoon.com)

b. Multigenic defense mechanism

Defense responses such as phytoalexin biosynthesis or lignin deposition in the cell wall require the action of many genes.

1. Peroxidases

Anionic peroxidases in the cell wall catalyze the production of phenolic radicals for the oxidative polymerization of lignin from cinnamyl alcohols. In tomato, there is

a marked induction of two linked genes encoding highly anionic peroxidases in an incompatible interaction with an avirulent form of *Verticillium albo-atrum*, with only weak induction in the compatible interaction with a virulent form of this vascular pathogen. Expression of one of these genes in transgenic tobacco under the control of either its own promoter or the CaMV 35s promoter resulted in massive increase in anionic peroxidase activity and these plants apparently showed a significant increase in resistance to *Peronospora parasitica* as judged by symptom development and fungal sporulation.

4. Activation of defense genes by chemicals

Several classes of compounds have the potential to act as inducers of natural resistance mechanisms in horticultural crops and chemicals with such indirect modes of action may offer attractive alternatives or supplement to existing contact/systemic fungicides in integrated disease management. Increase was found to occur in response to salicylic acid treatment as well as oligosaccharides and glycoproteins originating from either fungal cell wall or host cell walls called as elicitors. Recently, chitosan seed treatment has been found to induce defense related genes like chitinase and glucanase in tomato and consequently the *fusarium* crown and root rot diseases were significantly reduced. Pre-treatment with 2, 6-dichloroisonicotinic acid was highly effective in significantly reducing both anthracnose (*Colletotrichum lindemuthianum*) and rust (*Uromyces appendiculatus*) diseases in bean plants. (Agrimoon.com)

Modern approaches of breeding for disease resistance crops in plant pathology

1. Introduction
2. Selection
3. Hybridization followed by selection
4. Back cross method
5. Induced mutagenesis
6. Development of multilines
7. Cell and tissue culture
8. Biotechnological approaches

1. Introduction

It is a very simple and inexpensive method. Introductions have served as a useful method of disease control. It is possible that a variety resistant in one region need not be resistant in another region due to variation in the physiological race of the

pathogen or due to a much different agroclimatic condition in the new location. Their yield performance and disease resistance should be confirmed by large scale cultivation.

For example, Ridley wheat introduced from Australia - rust resistant variety. Manila, a rice variety introduced in Karnataka from the Philippines, has tolerance to blast, bacterial leaf blight and sheath blight. Intan, a Javanica type rice variety introduced in Karnataka from Indonesia is highly resistant to blast. Munal, a rice variety introduced in West Bengal from the U.S.A. is tolerant to blast, bacterial leaf blight and leaf folder (pest). Some of IRRI rice varieties such as IR 20, IR.24, IR.28, IR.34, IR.36 and IR .50 possess resistance to one or more diseases.

2. Selection

This is better method than introduction and has more chances of success in obtaining disease-resistant plants. The work of selection is carried out either in the naturally infected field conditions or under artificially inoculated conditions. The resistance in such individuals will occur in nature by mutation. To ensure the resistant character of a plant, large population of crop plant may be exposed to the attack of pathogen under artificial conditions and the non-infected plants may be chosen. Suvarnamodan rice of Kerala is a pure line of ARC is highly tolerant to blast. Sugandh of Bihar is a selection from Basmati rice of Orissa tolerant to bacterial leaf blight. Rice varieties Sudha (Bihar), Sabita, Nalini (West Bengal), Patel 85 (Madhya Pradesh), Janaki(Bihar), Improved White Ponni (Tamil Nadu), Ambika (Maharashtra), are some of rice selections resistant to one or more diseases.

3. Hybridization

When selection of resistant varieties is not feasible, resistant varieties may be evolved by crossing the susceptible popular variety with resistant wild variety where in the resistant gene or genes transferred into the genetic make up of susceptible variety. Very often the F1 from crosses may be resistant but carry the other undesirable qualities of the resistant parent. The bad qualities are removed by several back crossing of F_1 with the susceptible parent may ultimately yield a resistant progeny with good agronomic characteristics. Under certain circumstances pedigree or bulk method of selection is followed to obtain a resistant variety. Selection are made in F_2 generation for superior genetic traits including disease resistance. By continued selfing, selections are made through F_3 to F_5 or F_6 generations and the best variety is selected. This method is suited for small grains and beans but unsuited to fruits and vegetables.

4. Back cross method

Back cross method is widely used to transfer the disease resistance from wild species. Wild species are rice sources of disease resistance. Interspecific hybridization is made to transfer the gene or genes for resistance to the cultivated species. Resistance to grassy stunt virus from *Oryza nivara* to *O.sativa*, late blight resistance from *Solanum demissum* to cultivated potato, rust resistance from *durum* to *aestivum* wheat are some of the examples involving interspecific hybridization. Depending upon the number of genes governing resistance and the nature of the gene, whether dominant or recessive, the procedure varies. The number of back crosses to the cultivated species may be five to six. Once the back cross progeny resemble the cultivated parent, then they are selfed and segregating progeny screened for disease resistance.

5. Induced mutagenesis

While following mutation breeding for disease resistance, a large number of mutation progeny should be produced and screened under artificial epiphytotic condition to select resistant plants. MCU10 cotton, a resistant variety to bacterial blight was evolved in Tamil Nadu by subjecting seeds of a susceptible variety CO-4 to gamma rays followed by rigorous screening and selection

6. Development of multilines

The concept of multilines was first suggested by Jensen(1952) and developed by Borlaug (1959) for evolving multiline varieties to resist stem rust in wheat. A multiline variety is a composite of genetically similar lines, except that each line possesses a different gene for resistance to the pathogen. Lines that are genetically similar, except for one gene are called isoline. It is assumed that gene for resistance in each isoline contributes resistance to a separate physiological race or group of races. Genes for disease resistance are transferred by backcrossing from donor varieties to a common disease susceptible, but agronomically superior, recurrent parent. Isolines are generated differing only in the gene for disease resistance. The isolines are composited to synthesize a multiline variety. The isolines are maintained for resynthesizing the multiline whenever needed.

A multiline variety is composed of a mixture of resistant and susceptible genotypes and provides a buffering effect against rapid development of disease. It will provide resistance or tolerance to a broad spectrum of races of a pathogen. If new races of the pathogen are identified at a later stage, additional isolines resistant to the newly arisen races may be constituted and incorporated. Care should be taken to see that there is uniformity for height, maturity and other features in the multiline. Though multilines provide stability of yield due to reduction of damage by

pathogens, the limitations of multiline varieties are that the yield level of multiline varieties is limited to that of the recurrent parent, 4 to 5 years are required to stabilize isogenic lines and the pathogen may produce new races at a faster rate than the development of a multiline. Multiline varieties have been developed for resistance to stem rust and stripe rust of wheat and crown rust of oats. The first multiline variety in wheat- Miramar 60 was developed and released in Columbia to combat the attack of yellow rust. Miramar 63 and Miramar 65 were resistant to stem rust and stripe rust. Yoqui 50, Crew and Tumult are a few other wheat multilines. Kalyan sona and Sonalika-based multilines of wheat resistant to different races of rust have been developed in India.

7. Cell and tissue culture

Tissue culture approach is one of the best techniques in the field of molecular biology and it is applied in several ways for the development of disease resistance cultivars in agriculture and horticulture.

a. Somaclonal Variation

Selection *in vitro* aims to specific traits by subjecting large populations of cultured cells to the action of a selective agent in the petridish. For purpose of disease resistance, this selection can be done by fungal pathogens, culture filtrates of pathogens or isolated phytotoxins that are known to have a role in pathogenesis. The selection will allow only those cells to survive and proliferate that are resistant to the challenge. Plants regenerated from resistant cells often display a resistant phenotype when evaluated with either the toxin or the pathogen itself.

In order to be useful, new resistance traits, whether selected or not, must be heritable sexually or in the case of vegetatively propagated crops must be transmitted through vegetative propagules. The pathogens produced toxins can be used to screen calluses (cultured cells) which may regenerate resistant plants. The toxins will kill the calluses, but the mutant toxin resistant calluses will survive. The toxin-resistant calluses yield disease resistance plants. Similarly, *H. maydis* resistant maize plants, *H. sacchari* resistant sugarcane plants and *Phytophthora infestans* resistant tobacco plants have been evolved.

b. Anther culture

The plants are produced directly from microspores (immature pollen grains). Through anther or microspore culture, one has immediate access to unique and rare combinations of genes representing the recombination of the genetic material contributed by the parents of the cross. Through anther culture, followed by chromosome doubling, such

gene combinations can be fixed in their homozygous state as instant *inbreds* in a single step. Over the past two decades, anther culture has become widely accepted as a tool in cultivar development. This technique can be particularly useful for producing plants with novel combinations of resistance genes for managing fungal diseases.

c. Protoplasmic fusion

This generates hybrid cells by merging the total cellular components of somatic cells from which the cell walls have been removed to produce protoplasts. The incompatibility preventing sexual fertilization between species is thus avoided and viable hybrids have been created, even between unrelated distance species. Disease resistance genes have been transferred by protoplasts fusion from wild species into potato.

Disease resistant plants from tissue culture (Agrimoon.com)

S.no	Plant	Culture system	selection	Resistance to pathogen
1.	Potato	Protoplasts	SCV	*Phytopthora infestans Alternaria solani*
		Callus	CF	*Fusarium oxysporum*
2.	Tomato	Callus	Fusaric acid	*Fusarium oxysporum*
		Protoplasts		
3.	Banana	Meristem	SCV	*Fusarium oxysporum*
4.	Strawberry	Callus	SCV	*Fusarium oxysporum*

8. Biotechnological Approaches

1. Genetic Engineering

Genetic Engineering is the technology by which a particular gene is isolated from one organism and inserted into the genome of another organism and made to express at the right time.

Vectors for transfer of genes

Genetic engineering has been used to manage plant virus diseases. For transfer of genes to plants vectors are needed in which the gene to be transferred will multiply several folds. The most effective gene vector developed is the Tumour inducing plasmid of *Agrobacterium tumefaciens* from which the Tumor inducing genes have been removed *A.tumefaciens* induces tumors (crown galls) through ti-plasmid (tumor-inducing) which is a circular double stranded DNA molecule containing up

to 2,00,000 base pairs organized into several genes. The Ti-plasmid is transferred from the bacterium into the cell. A specific region of the plasmid, the T-DNA, is transferred from the plasmid to the nucleus of the plant cell. It becomes integrated into the plant nuclear genome and transcribed. Cauliflower mosaic virus (CaMV) is the only plant virus with double-stranded DNA genome. As it has DNA genome, it is used as a possible vector in introducing foreign genes into plant. It is possible to insert a non-viral gene into CaMV genome and obtain expression of the gene in the infected plant. The viral promotor regions from CaMV are effective for obtaining expression of other genes in plant cells. The genes to be expressed is now fused to a promotor element from CaMV and a gene of *A.tumifaciens*. They are then introduced into the plants using *A. tumefaciens* Ti-DNA transformation.

DNA construction

Messenger RNA is extracted and exposed to an enzyme reverse transcriptase which synthesizes a complimentary single stranded DNA. The complimentary DNA (cDNA) is exposed to another enzyme, DNA polymerase, which produces the double stranded cDNA. The cDNAs are inserted into the plasmids of *A. tumefaciens*.

2. Transgenic Plant Disease Management

1. Mitochondrial RNA (MIC RNA) expression in transgenic plants

A DNA copy is made of one or more sections of the viral genome that include the initiation codon for vital proteins to virus replication. The DNA copy is inserted in the host-cell genome, Cells then produce an `antisera RNA' called mic RNA (mRNA interfering complementary to 5' end of the gene). The mic RNA hybridizes *in vivo* with the viral mRNA blacking translation. The mic RNA is inserted into the plants using the Ti plasmid of *A. tumefaciens*. Plants regenerated from the transformed cells will be resistant to the particular virus. This possibility is also being exploited for the control of virus diseases.

2. Use of DNA markers for cloning resistance genes

Molecular markers *viz*., isozymes and DNA markers (Restriction Fragment Length Polymorphisms - RFLPs; Random Amplified Polymorphic DNA - RAPD and others) are being used in several areas relevant to identification of disease resistance genes. Some of the disease resistance genes using random DNA markers have been identified.

Disease resistance genes mapped using RFLP markers

Plant	Pathogen
Tomato	*Fusarium oxysporum*
citrus	*Phytopthora* spp

3. Satellite RNA expression in transgenic plants

Satellite RNAs are associated with several viruses. They are packaged into virus particles along with the genomic RNAs of the helper virus. They are not part of the viral genome and have no obvious sequence relationships with the helper virus. The presence of the satellite RNA suppresses the disease severity in many hosts. Hence transgenic plants which express satellite RNA have been produced to manage virus diseases. e.g., Transgenic plants of tobacco expressed the synthesis of satellite tobacco ring spot virus and reduce the virus disease incidence. Satellite RNA expressing tobacco plants against Cucumber Mosaic Virus (CMV) and Tobacco aspermy virus have been synthesized.

4. Candidate Genes

a. Viral Pathogens

Use of transgenic resistance against plant disease is that was accomplished in the management of papaya ring spot virus (PRSV) in Hawaii (Jain, 1993). Coat- protein-mediated resistance using coat protein genes sourced form a Hawaiian strain of PRSV was attempted. One transgenic line was found to be completely resistant to PRSV (Mandahar and Khurana, 1998; Balasubramania, 2009).

Recently, a gene silencing mechanism has been put to productive use in obtaining rice yellow mottle virus. An open reading frame of the virus itself is expressed in rice in order to stop the viral spread in an effective manner. Similar attempts also have been made in obtaining multiple viral infections (tomato spotted wilt virus and turnip mosaic virus) in plant (Chopra and Sharma, 1991).

b. Bacterial Pathogens

A wide-spectrum bacterium bacterial blight resistance gene Xa-21, sourced from an African rice, *Oryza longistaminata* was backcrossed into cultivated variety by scientists of the International Rice Research Institute, the Philippines (IRRI). The resistance gene was cloned using molecular means by Pam Ronald of University of California and distributed to labs all over the world, so that the gene could be put into rice cultivars of local importance (Oswald, 1951).

Wild Fire disease of tobacco caused *Pseudomonas syringae* pv. *tabaci* is a serious disease. A phytotoxin secreted by the pathogen drastically modifies the amino acid metabolism of the plant with the eventual accumulation of ammonia in tobacco leaves, which causes extensive blighting (Maloy, 2005). Interestingly, the pathogen that synthesis the phytotoxin remains unaffected by the toxin. This formed the basis for a search of the candidate gene from the pathogen itself.

A toxin-inactivating gene, which was named 'ttr' was successfully isolated from the pathogen and the same was cloned into tobacco cultivars which showed excellent wildfire resistance (Agrios, 1988).

c. Fungal Pathogens

PR- protein genes appear to be a very potential source for candidate genes for fungal resistance. These proteins may play a direct role in defense by attacking and degrading pathogen cell wall components. Typical candidate genes are that encoding chitinases and ß – 1, 3 glucanases (Fuchs and Gonsalves, 1996) increasing expression of individual and multiple PR-proteins in various crops have demonstrated some success in enhancing disease resistance in particular pathogens (e.g. Rice against *Rhizoctonia solani*, the shealth blight pathogen). A result of a research shows a chitianase gene from an anti-fungal bio-control fungus species (*Trichoderma viridae*) confers transgenic resistance against the rice sheath blight pathogen. A rice PR-5 protein gene in wheat delays onset of symptoms caused by wheat scab pathogen (Maloy, 2005).

Conclusion

We have to control the plant diseases that have plagued mankind since the dawn of agriculture. The biotechnological methods to combat disease reviewed here are more effective, environmentally friendly and safer than many current common methods of control. We need to double our food production by 50 years, and 70% of this increase needs to be achieved by adopting new technology. Therefore, we cannot ignore these approaches. However, almost the people in the growing decades needs ultimate disease free food and atleast maintaining the healthy and good soil fertility.

References

Agrios, G. N. (1988). Plant pathology *3rd Ed. (1997) 4th(Ed.)* New York (pp123-178). *Elsevier Academic Press.*

Agrios, G. N. (2005*).* Plant Pathology. *5th(Eds.),* New York (pp. 213-223). *Elsevier Academic Press*

Agnihotri, V. P. N., Singh, H. S., Chaube, U. S., Singh and Dwivedi, T. S. (1989). Perspectives in plant pathology. *Today and Tomorrow printers and publishers*, New Delhi, India.

Balasubramania (2009). Biotechnology centre for plants molecular Biology. Jamel Nadu Agricultural University, Coimbatore, India.- 641003

Chopra, V. L. and R. P. Sharma. (1991). Biotechnology in crop improvement. *Current science,* 60(9 and 10), 443-547

Cook, R. J. and Baker, K. F. (1983). The native and practice of biological control of plant pathogens. *Annals of phytopathology Society*., *St paul, Minn*., USA.

Fuchs, M. and Gonsalves, D., (1996). Genetic engineering. In: environmentally safe Approaches to crop disease control, *N.A. Rechcigl and J.E. Rechcigl (Eds)* BocaRaton, Newyork, (pp. 333-368). *CRC Press.*

Jain, S. M. (1993). Recent advances in plant genetic engineering. *Current Science,* 64(10), 715- 724

Maloy, O. C. (2005). Plant disease management. *The plant instructor.*

Mandahar, C. L. and Khurana, S. M. P. (1998). The role of plant biotechnology in controlling plant diseases in: Pathological problems of economic crop plants and their management, *S. M. Paul Khurana (ed.),* Jodhpur, India, *Scientific Publishers*. (pp. 637-647)

Oswald. J. W. (1951). The relation of periconia to milo root in califonia, *Phytopath*; 41, pp-28 – 29

www.Agrimoon.com

17 Molecular Techniques in Plant Pathology: Advances and Applications

***R. L. Joshi[1], J. J. Padsala[1], C. M. Bhaliya[2] and H. A. Shekhada[3]**

[1]Ph. D. Scholar, Department of Plant Pathology, Navsari Agricultural University (Gujarat) 396 450.
[2]Assistant professor, Department of Plant Pathology, Junagadh Agricultural University (Gujarat) 362 001.
[3]Ph. D. Scholar, Department of Plant Pathology, Junagadh Agricultural University (Gujarat) 362 001.

Abstract

Molecular techniques have revolutionized the field of plant pathology by providing powerful tools for the detection, diagnosis and management of plant pathogens. These techniques offer several advantages over traditional methods, including higher sensitivity, specificity and speed. Molecular methods such as DNA barcoding, DNA/RNA probe methods, in situ hybridization, microarray, Loop-mediated isothermal amplification (LAMP) and CRISPR/Cas9 genome editing have greatly advanced our understanding of plant diseases and opened new avenues for disease control. They facilitate early detection of diseases, providing crucial information for timely intervention and preventing further spread. These molecular techniques enable the accurate identification of plant pathogens including bacteria, fungi, viruses and oomycetes, allowing for targeted management strategies. The application of molecular techniques in plant pathology has not only improved disease diagnostics but also enhanced breeding programs for developing resistant crop varieties. By identifying and targeting specific genes associated with disease resistance, researchers can utilize genetic engineering tools to enhance plant defence mechanisms and reduce crop losses.

Keywords: Molecular techniques, detection, diagnosis, plant pathogen

Introduction

The degradation of plant products, which results in a global loss estimated between 10 and 30 percent, poses a significant constraint on food resources (Okawa, 2015). Pathogens and their associated toxins are major contributors to this deterioration, making food safety a critical concern. Bacterial, fungal, and viral infections are prominent culprits behind plant diseases, spreading extensively throughout plantations through the introduction of disease-causing agents or infected plants (Sankaran *et al.*, 2010). In both fundamental and applied plant research, early assessment of disease occurrence and severity in field crops is vital (Gautam and Kumar, 2020). Accurate and timely assessments are necessary as they form the basis for implementing effective plant protection measures in the field.

Plants are susceptible to infections caused by various pathogenic microorganisms throughout their lifecycle, starting from seedling emergence until maturity. Microbial pathogens pose a significant threat to crop production worldwide, leading to substantial losses. Infectious diseases caused by these pathogens continue to hinder efforts aimed at increasing agricultural output globally. As a result, monitoring plant health and promptly diagnosing plant diseases are essential for mitigating the extent of damage caused by pathogens. Early detection of diseases provides valuable information that facilitates the implementation of effective and precise management strategies to control and mitigate the impact of these diseases.

Globally, the agricultural sector suffers significant economic losses due to various plant pathogens. These escalating agricultural losses have captured the interest and concern of researchers and experts, leading to a strong emphasis on the advancement of miniaturized systems for pathogen detection and management in the field of phytopathology.

Traditional *vs* Molecular methods

Traditional methods and molecular methods are two different approaches used in plant pathology for the detection, identification, and management of plant diseases. Here are some key differences between these two methods:

1. **Principle of Detection:**
    - » Traditional methods: Traditional methods rely on visual observation of symptoms, morphological characteristics, and growth patterns of pathogens or their effects on plants.
    - » Molecular methods: Molecular methods involve the detection and analysis of specific nucleic acids (DNA or RNA) of the pathogens using techniques such as PCR, DNA sequencing, or hybridization assays.

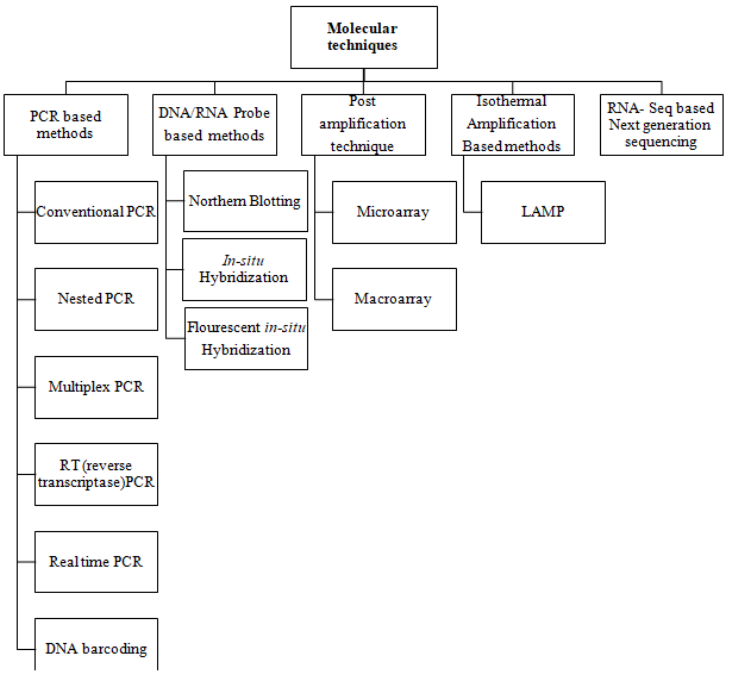
2. **Sensitivity and Specificity:**
   - » Traditional methods: Traditional methods may lack sensitivity, especially in early stages of infection or when pathogens are present in low concentrations. They also have limitations in distinguishing closely related pathogens or strains.
   - » Molecular methods: Molecular methods offer high sensitivity, allowing detection of pathogens even at low concentrations. They provide high specificity by targeting specific genetic markers, enabling differentiation of closely related pathogens or strains.
3. **Speed and Efficiency:**
   - » Traditional methods: Traditional methods often require time-consuming processes, such as culturing pathogens, observing symptoms over time, or performing labour-intensive diagnostic tests.
   - » Molecular methods: Molecular methods offer rapid and efficient detection, allowing for quicker diagnosis and decision-making. Techniques like PCR can produce results within hours.
4. **Accuracy and Reliability:**
   - » Traditional methods: Traditional methods heavily rely on the expertise and experience of pathologists, which can introduce subjectivity and variability in the diagnosis.
   - » Molecular methods: Molecular methods provide objective and reproducible results, minimizing subjective interpretation. They offer high accuracy and reliability due to the specificity of nucleic acid-based detection.
5. **Pathogen Characterization:**
   - » Traditional methods: Traditional methods provide limited information about the genetic characteristics and diversity of pathogens.
   - » Molecular methods: Molecular methods allow for detailed characterization of pathogens at the molecular level, including genetic diversity, strain identification, and evolutionary relationships.
6. **Disease Management:**
   - » Traditional methods: Traditional methods inform disease management based on visual symptoms and general knowledge of pathogen behaviour.
   - » Molecular methods: Molecular methods enable targeted and precise disease management strategies by providing early detection, identification of specific pathogens/strains, and insights into pathogen populations and dynamics.

Molecular techniques for detection of plant pathogens:

The molecular detection and diagnosis of fungal plant pathogens require specific pre-analytical steps, including the efficient lysis of fungal cells and the recovery of genomic DNA, as well as the subsequent purification and quantification of the extracted DNA. Various protocols for DNA isolation from fungi that infect plants have been developed. Recently, commercial DNA extraction kits have also been employed in fungal plant disease diagnostics (Martinelli *et al.*, 2015; Moffat *et al.*, 2015).

However, many laboratories still rely on standard protocols, which involve steps such as lyophilization of fungal mycelia, disruption of the chitin cell wall through grinding, and subsequent DNA isolation in a buffer containing chemicals. These protocols also include the removal of proteins using a phenol-chloroform mixture and DNA precipitation with propanol. The isolated fungal DNA can then be further purified using standard methods, including pelleting, silica membrane (Mancini *et al.*, 2016), spin filter, or silica-coated magnetic particle separation. Finally, the concentration of fungal DNA in the samples can be determined using a UV spectrophotometer (Abdullah *et al.*, 2018).

Fig 1: Flow chart of molecular techniques used for the detection of plant pathogens (Aslam *et al.*, 2017)



PCR:

Polymerase Chain Reaction (PCR) is a widely used molecular technique that allows for the amplification of specific DNA sequences. In plant pathology, PCR has been instrumental in the rapid and accurate detection of plant pathogens. Pathogen-specific primers designed to amplify unique regions of the pathogen's DNA, enabling its identification even at low levels in infected plant tissues or environmental samples. PCR-based techniques, such as real-time PCR and multiplex PCR, have further improved the speed and specificity of pathogen detection.

Table 1: PCR-based techniques used in the diagnosis of various pathogenic fungi

Assay	Diagnosed fungi	Host	Disease	Target gene	Reference
End-point PCR	*Cercospora tezpurensis sp. nov.*	*Capsicum assamicum*	Leaf spot	ACT, CAL, HIS and TEF-1a	Meghvansi *et al.*, 2013
End-point PCR	*Exobasidium maculosum*	Blueberry	Leaf and fruit spot	LSU-rDNA	Brewer *et al.*, 2014
End-point PCR	*Golovinomyces cichoracearum sensu lato*	*Cannabis sativa*	Hemp powdery mildew	ITS	Pépin *et al.*, 2018
End-point PCR	*Cercospora cf. flagellaris*	*Cannabis sativa*	Hemp leaf spot	ITS, TEF-1a, CAL, HIS and ACT	Doyle *et al.*, 2019
End-point PCR	Neopestalotiopsis clavispora and *Colletotrichum siamense*	Macadamia	Leaf spot	ITS, TUB2, TEF-1a, ACT and GAPDH	Prasannath *et al.*, 2020
Nested PCR	*Puccinia striiformis f. sp. tritici*	Wheat	Stripe rust	PSR	Wang *et al.*, 2009
Nested PCR	*Phytophthora cactorum*	Strawberry	Crown rot	ITS	Bhat and Browne, 2010
Nested PCR	*Colletotrichum gloeosporioides*	*Dioscorea* spp.	Greater yam anthracnose	ITS	Raj *et al.*, 2013
Nested PCR	*Pilidiella granati*	Pomegranate	Twig blight and crown rot	SSU-rDNA	Yang X. *et al.*, 2017

Multiplex PCR	Fusarium Verticillioides and F. subglutinans	Maize	Stalk rot and ear rot	gaoB	Faria *et al.*, 2012
Multiplex PCR	Neofabraea alba, N. perennans and *N. keinholzii*	Apple	Bull's eye rot	TUB2	Michalecka *et al.*, 2016
Multiplex PCR	Fusarium oxysporum f. *sp. cubense lineage VI strains*	*Musa* spp.	Dessert/beer bananas	TEF-1a and RPC2	Ndayihanzamaso *et al.*, 2020
Quantitative PCR	*Didymella bryoniae*	Cucurbits	Gummy stem blight	RAPD	Ling *et al.*, 2010
Quantitative PCR	*Ramularia collo-cygni*	Barely	Ramularia leaf spot	Not mentioned	Havis *et al.*, 2014
Quantitative PCR	*Rhizoctonia solani*	Tobacco	Target spot	ITS	Zhao Y. Q. *et al.*, 2014
Quantitative PCR	*Magnaporthe oryzae*	Rice	Rice blast	18S-28S rDNA	Sun *et al.*, 2015
Quantitative PCR	*Verticillium longisporum*	*Brassica napus*	Wilt and stem stripe	TUB2	Depotter *et al.*, 2017
Quantitative PCR	Pyrenophora tritici-repentis and *Parastagonospora nodorum*	Wheat	Tan (yellow) spot and Septoria blotch	ToxA	Abdulla *et al.*, 2018
Quantitative PCR	*Fusarium culmorum*	Cereals	Foot and root rot and Fusarium head blight	COX2	Bilska *et al.*, 2018
Quantitative PCR	*Fusarium guttiforme*	Pineapple	Fusariosis	TEF-1a and TUB2	Carnielli-Queiroz *et al.*, 2019
End-point PCR and quantitative PCR	Phacidiopycnis washingtonensis and *Sphaeropsis pyriputrescens*	Apple	Speck rot and Sphaeropsis rot	ITS	Sikdar *et al.*, 2014
End-point PCR and quantitative PCR	*Guignardia citricarpa*	Citrus spp.	Citrus black spot	ITS	Faganello *et al.*, 2017

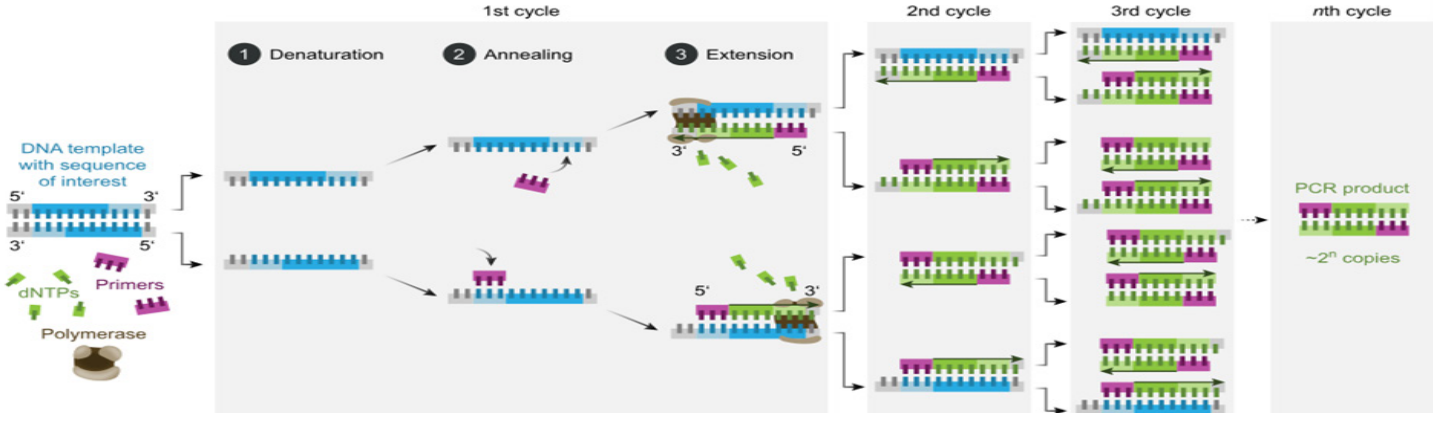


Fig 2: Schematic drawing of a complete PCR cycle (https://en.wikipedia.org/wiki/Polymerase chain reaction)

DNA barcoding:

DNA barcoding is a molecular diagnostic technique that utilizes a small segment of DNA to identify the species across various domains of eukaryotic life. This approach involves using standardized sequences of 500-800 base pairs as markers for species identification (Krishnamurthy and Francis, 2012). Several barcoding markers, such as ITS, SSU, LSU, EF-1α, and RPB, have been employed for fungal DNA barcoding. ITS (Internal Transcribed Spacer) commonly used as a universal barcode region in fungal DNA barcoding (Schoch *et al*., 2012; Li *et al*., 2011). The high level of polymorphism observed in the ITS region makes it a robust and reliable candidate for fungal DNA barcoding. However, in cases where primary barcodes are insufficient for identifying pathogenic fungal species, secondary barcodes developed to enhance accuracy. In the context of plant DNA barcoding, the ITS-2 spacer has proven to be a highly informative secondary barcode (Xu, 2016). This region offers valuable insights into species identification and classification, particularly in cases where primary barcodes are less effective.

ADVANTAGES

DNA barcoding offers numerous scientific advantages, including:

- Facilitating species identification: DNA barcoding allows for the identification of species at any life stage, even when traditional morphological identification is challenging or impractical. This is particularly useful for identifying species based on their DNA sequences.
- Species discovery through phylogenetic analysis: DNA barcoding enables the discovery of new species by analyzing their nucleic acid sequences and comparing them to known species. Phylogenetic analysis helps in understanding the evolutionary relationships and diversification of life.
- Advancing DNA sequencing tools for biodiversity research: DNA barcoding has contributed to the development and improvement of DNA sequencing technologies and tools. These advancements have been instrumental in studying biodiversity on a large scale, enabling researchers to analyze and compare DNA sequences efficiently.

DNA/RNA probe-based methods :

DNA/RNA probe methods offer highly sensitive and rapid diagnostic capabilities for plant diseases caused by microbial pathogens. These methods involve the use of probes to analyze nucleic acids without the need for amplification. Probes are short, single-stranded DNA sequences that are labelled with chemiluminescent reporter

molecules or radio-labelled isotopes like 32P, 33P, or 35S. These labelled probes used to identify specific homologous sequences on the target DNA. Traditionally, DNA probes employed as an alternative to PCR for the identification of fungi in plant pathology. However, in recent methods, DNA probes often used in combination with PCR techniques (McCartney *et al.*, 2003). This combination approach enhances the sensitivity and specificity of detection by using the highly specific nature of DNA probes to target specific nucleic acid sequences, while PCR amplifies the targeted DNA to increase the signal strength.

Northern blotting:

The Northern blot is a method used to transfer RNA onto a carrier for the identification of fungi. This process involves several steps:

1. RNA Purification: Firstly, RNA purified from each tissue to examine the expression of the gene of interest.
2. Gel Electrophoresis: The purified RNA is then loaded onto an agarose gel. An electric current applied to the gel, causing the RNA molecules to move towards the bottom. Smaller RNA molecules move faster than larger ones, leading to separation based on size.
3. Transfer to Filter: After gel electrophoresis, the separated RNA fragments blotted onto a special filter paper, where each RNA molecule maintains its relative position to all other molecules.
4. Hybridization with Probes: The filter is then exposed to radioactive probes that are complementary to specific RNA sequences. The probes hybridize with their complementary RNA sequences on the filter.
5. Autoradiography: After hybridization, the filter is subjected to autoradiography to develop the film. If the probe has successfully hybridized to a fragment of RNA on the filter, a band will be detected on the autoradiograph.

The Northern blotting technique is useful for studying gene expression. However, it has relatively lower sensitivity compared to the modern RT-PCR (Reverse Transcription Polymerase Chain Reaction) technique. Both real-time PCR and Northern blotting used to identify *Magnaporthe grisea* in rice plants (Qi and Yang, 2002).

***In situ* Hybridization:**

The in situ hybridization (ISH) technique utilizes single-stranded RNA probes that labelled with 35S. In ISH, various types of probes can be used, including synthetic oligonucleotides, cDNAs, and cRNAs. However, probing with riboprobes tends to yield the most sensitive and efficient results. Among the radioactive probes, 35S-labeled

riboprobes are particularly sensitive and commonly used for the identification of mRNA (Hayden *et al*., 2002). ISH is a valuable tool for visualizing the infection of plant tissues by rust fungi.

Fluorescent *in-situ* Hybridization (FISH):

FISH is a powerful technology in plant disease diagnostics, enabling the specific detection and visualization of DNA or RNA sequences in plant cells or tissues. It combines the specificity of DNA sequences with the sensitivity of fluorochrome-based detection systems.

Post Amplification Technique

Microarray:

Microarray technology has emerged as a highly efficient system for analysing large-scale gene expression patterns simultaneously. The fundamental principle of DNA microarrays based on the ability of complementary sequences to bind to each other through hybridization. This specific binding allows target DNA or RNA molecules to hybridize with specific complementary DNA probes that immobilized on the microarray. The microarray consists of an array of DNA probes, each representing a specific gene or sequence of interest. These probes attached to a solid surface, such as a glass slide or silicon chip. When a labelled target DNA or RNA sample applied to the microarray, the complementary sequences present in the sample will hybridize with their corresponding DNA probes on the array.

This technique has successfully identified the presence of *Aspergillus candida* and Mycobacterium (Singh and Kumar 2013). Furthermore, four species of cucurbit-infecting tobamoviruses were detected using viral microarray technology. In this case, a plant virus cDNA chip was designed, and a manual spotting system was employed to immobilize viral cDNA probes onto the chip. This enabled the simultaneous detection and identification of multiple tobamovirus species in cucurbit plants. The combination of hybridization and PCR amplification in this technique allows for the selective binding of target DNA or RNA to specific complementary probes, resulting in the identification and analysis of the desired microorganisms or viral species. This approach is highly effective in detecting and characterizing pathogens in various plant samples, contributing to the field of plant disease diagnostics and management.

Macroarray:

The Macroarray or DNA array hybridization, also known as Reverse dot plot, is a sensitive technique for identifying microbial species without the need for

radioisotopes (Singh and Kumar, 2013). It utilizes the power of DNA amplification and hybridization simultaneously. This method involves the simultaneous PCR amplification of related species and the subsequent hybridization with specific DNA probes. By combining PCR with hybridization, the sensitivity of this assay significantly increased, up to 1000-fold or even higher compared to traditional PCR methods. This improved sensitivity enables the detection and identification of microbial species with greater precision and accuracy. The Macroarray technique successfully applied for the identification of various pathogenic microorganisms, including *Alternaria alternata, Aspergillus fumigatus, Fusarium solani, Candida albicans* and *Cladosporium herbarum* (Sato *et al*., 2010). Additionally, it has been used for the identification of fungal and oomycete pathogens that cause diseases in solanaceous crops (Zhang *et al*., 2008).

Isothermal amplification-based methods

Loop-mediated isothermal amplification

The Loop-mediated isothermal amplification (LAMP) technique is a nucleic acid amplification method that allows for the rapid and specific amplification of target DNA sequences under isothermal conditions. The LAMP reaction involves several steps, which summarized as follows:

1. Primer design: Design specific primers that recognize multiple regions within the target DNA sequence. These primers typically consist of two outer primers (forward and backward) and two inner primers (forward inner and backward inner) that initiate the amplification process.
2. Reaction setup: Prepare a reaction mixture containing template DNA, LAMP primers, Bst DNA polymerase, nucleotides, buffer solution, and MgSO4.
3. Incubation: Incubate the reaction mixture at a constant temperature (around 60-65 degrees Celsius) for amplification.
4. Amplification process: The LAMP reaction involves strand displacement, auto-cycling strand displacement, accumulation of DNA, and exponential amplification.
5. Detection: Visualize the amplified DNA using colour change, turbidity, real-time monitoring, or gel electrophoresis.

The LAMP (Loop-mediated isothermal amplification) method has successfully been utilized for the diagnosis of *Ascochyta rabiei* L., *Penicillium marneffei*, and *Ophiostoma clavatum* (Sun *et al*., 2010). This technique offers a rapid and sensitive approach for detecting and identifying these specific microorganisms.

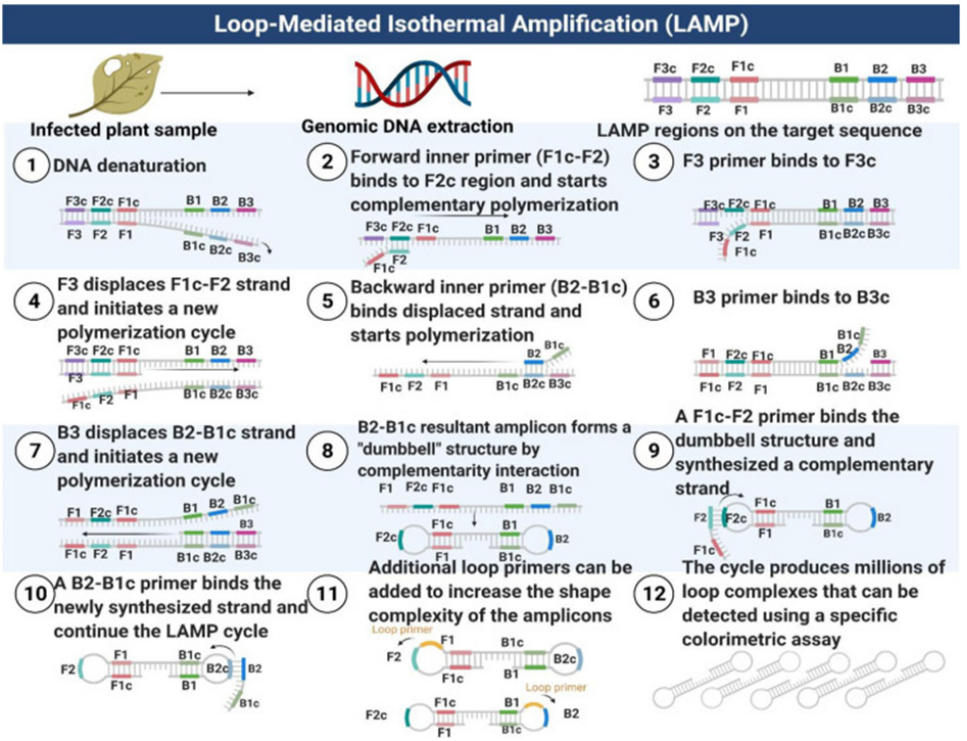


Fig 3: Principle of LAMP assay (Patel *et al.*, 2022)

Next-Generation Sequencing (NGS):

Next-generation sequencing (NGS) technologies have revolutionized the field of genomics by providing high-throughput sequencing capabilities. NGS enables the sequencing of entire pathogen genomes, transcriptomes, and metagenomes. In plant pathology, NGS has been instrumental in identifying novel pathogens, studying pathogen populations, and unravelling host-pathogen interactions. RNA-Seq, a transcriptomic approach using NGS, allows for the identification and quantification of differentially expressed genes during infection.

Molecular techniques for plant disease management

RNA interference

RNA interference (RNAi) is a cellular process that regulates gene expression by silencing specific genes. The steps of RNA interference are as follows:

1. dsRNA Formation: Double-stranded RNA (dsRNA) is introduced into the cell, either by exogenous delivery or through the transcription of a specific gene to produce dsRNA.

2. Dicer Cleavage: An enzyme called Dicer recognizes the dsRNA and cleaves it into small interfering RNAs (siRNAs) that are typically 21-25 nucleotides long.
3. RISC Assembly: The siRNAs are then loaded onto the RNA-induced silencing complex (RISC), where one of the siRNA strands, known as the guide strand, is selected.
4. Target Recognition: The guide siRNA within the RISC complex binds to the complementary mRNA sequence of the target gene.
5. mRNA Degradation: The binding of the guide siRNA to the target mRNA triggers its degradation or prevents its translation into a functional protein
6. Gene Silencing: The targeted gene effectively silenced, leading to a reduction or elimination of the corresponding protein's expression.

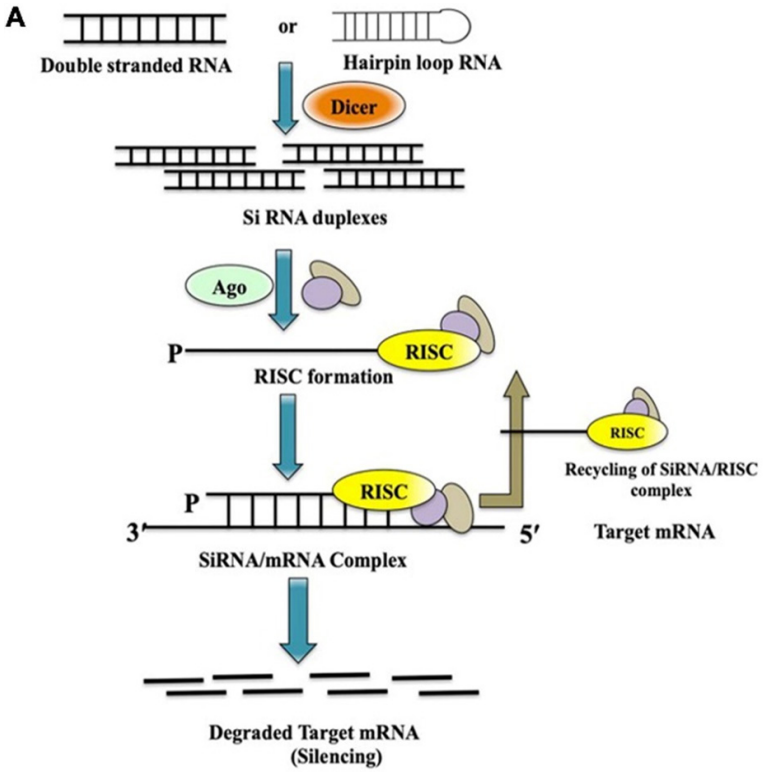


Fig 4: RNAi-mediated gene silencing (Halder *et al.*, 2022)

Table 2: RNAi-targeted editing in plants against fungi (Halder *et al.*, 2022)

Fungi	Target gene	Plant	Phenotype
Puccinia triticina	PtMAPK1	Wheat	Significant reduction of fungal growth and disease suppression
Blumeria graminis f.sp. *tritici*	MLO	Wheat	Inhibition of fungal growth
F. graminearum	IRT containing mycotoxin regulatory sequences	Grain and legume crops	Significant reduction in mycotoxin production
Blumeria graminis	Avra10	Barley and wheat	Inhibition of fungal growth
Phytophthora parasitica	PnPMA1	Arabidopsis	Elucidation of a compatible interaction between Arabidopsis and *P. parasitica*
Fusarium verticillioides	GUS	Tobacco	Reduced expression of *GUS*
Phytophthora parasitica var. nicotianae	Glutathione S-transferase	Tobacco	Increase resistance of Nicotiana to infection
Puccinia striiformis f. sp. *tritici*	PSTha12J12	Barley and wheat	Significant improvement in rust resistance
Botrytis cinerea	MAP Kinase *Bmp3*	Lettuce	Delay in conidial germination, reduction of necrotic lesions
Botrytis cinerea	*DCL1*, *DCL2*	Tomato, Strawberry, Grape, Lettuce, Onion, Rose	Significant inhibition in gray mold disease
Botrytis cinerea	DND1	Tomato and Potato	Reduced susceptibility to Botrytis
Botrytis cinerea	BcTOR	Arabidopsis, Potato, Tomato	Enhanced resistance against gray mold

Genome Editing:

In recent times, advanced breeding technologies like meganucleases (MNs), zinc-finger nucleases (ZFNs), transcription-activator-like (TAL) effector nucleases (TALENs), and clustered regularly interspaced palindromic repeats (CRISPR) have emerged. Among these, the CRISPR/Cas9 system has gained significant attention for enhancing disease resistance in various crops such as rice, cacao, wheat, tomato, and grape. This system enables precise editing of the genome through the activity of RNA-guided DNA endonucleases. Apart from crop genome editing, this technology has also shown promise in editing the genomes of fungal and oomycete pathogens, providing novel strategies for plant disease management. CRISPR/Cas tools have been successfully used to combat oomycete diseases by targeting specific genes, such as the PAMP-triggered immunity repressor AtERF019 (ethylene-responsive factor 19 gene), leading to increased resistance against *Phytophthora parasitica* in *Arabidopsis thaliana*. Applications of genome editing in developing disease-resistant plants, overcoming disease susceptibility factors and engineering plant defense mechanisms. (Paul *et al.*, 2021)

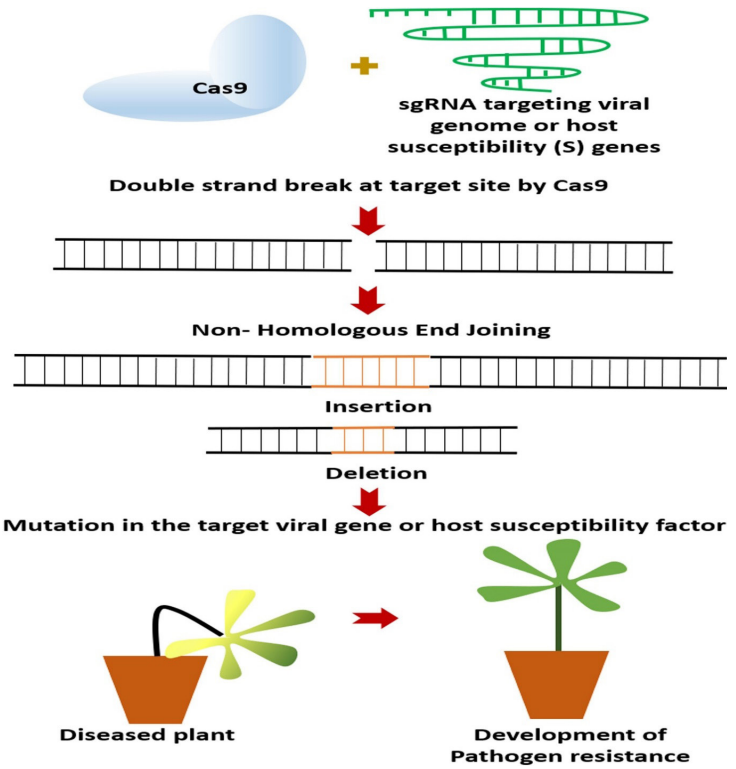


Fig 5: The CRISPR/Cas9 targeting and subsequent non- homologous end joining (NHEJ) process exploited for development of pathogen resistance in plants (Das *et al.*, 2019).

Table 3: Genome editing technologies for disease resistance in plants. (Yin and Qiu 2019)

DISEASE	TARGET GENE	NUCLEASE	MUTATION	PATHOGEN	HOST PLANT
Bacteria					
rice bacterial blight	*OsSWEET13*/exon	CRISPR/ Cas9	deletions	*Xanthomonas oryzae* pv. *oryzae*	rice
bacterial speck, *phytophthora* blight, bacterial spot	*SlDMR6-1*/exon	CRISPR/ Cas9	deletions	*Pseudomonas syringae* pv. *tomato*, *Phytophthora capsici*, *Xanthomonas* spp.	tomato
citrus canker	*CsLOB1*/exon	CRISPR/ Cas9	In-dels	*Xanthomonas citri* subsp. *citri*	citrus
Fungi					
powdery mildew	*TaMLO*/exon	TALEN	In-dels	*Blumeria graminis* f. sp. *tritici*	wheat
powdery mildew	*TaEDR1*/exon	CRISPR/ Cas9	In-dels	*Blumeria graminis* f. sp. *tritici*	wheat
powdery mildew	*SlMlo1*/exon	CRISPR/ Cas9	deletions	*Oidium neolycopersici*	tomato
Viruses					
DNA viral disease	replication origin	AZP	n.d.	BSCTV	*Arabidopsis*
DNA viral disease	Rep	ZFN	n.d.	TYLCCNV and TbCSV	tobacco
DNA viral disease	Rep	TALE	n.d.	TbCSV, TYCCNV and TLCYnV	tobacco
DNA viral disease	IR, CP, *RCRII*	CRISPR/ Cas9	In-dels	TYLCV, BCTV, MeMV	tobacco
DNA viral disease	LIR, Rep	CRISPR/ Cas9	In-dels	BeYDV	tobacco
DNA viral disease	IR, CP, Rep	CRISPR/ Cas9	In-dels	BSCTV	tobacco and *Arabidopsis*
DNA viral disease	IR, CP, Rep	CRISPR/ Cas9	In-dels	CLCuKoV,TYLCV, TYLCSV, MeMV, BCTV-Logan, BCTV-Worland	tobacco
RNA viral disease	*eIF4E*/exon	CRISPR/ Cas9	deletions	CVYV, ZYMV, PRSV-W	cucumber
RNA viral disease	*eIF(iso)4E*/exon	CRISPR/ Cas9	In-dels	TuMV	*Arabidopsis*

RNA viral disease	*nCBP-1&nCBP-2/ exon*	CRISPR/ Cas9	In-dels	CBSV	cassava
RNA viral disease	ORF1a, ORFCP and 3'-UTR	CRISPR/ Cas9	no cleavage	CMV	tobacco and *Arabidopsis*
RNA viral disease	GFP, Hc-Pro and CP	CRISPR/ Cas13a	n.d.	TuMV	tobacco

Conclusion

In the field of plant pathology, molecular techniques provided powerful tools for the detection, diagnosis and management of plant pathogens. Molecular methods have played a crucial role in the accurate identification of pathogens at the species or strain level, which is essential for disease surveillance and the development of targeted control measures. These techniques provided valuable insights into the genetic diversity, evolution and virulence factors of these pathogens. Such knowledge has facilitated the development of more effective disease management strategies. These advancements hold great promise for improving agricultural productivity and ensuring food security. By harnessing the power of molecular technologies, we can create a more resilient and sustainable agricultural system for the future.

References

Abdullah, A. S., Turo, C., Moffat, C. S., Lopez-Ruiz, F. J., Gibberd, M. R. and Hamblin, J. (2018). Real-time PCR for diagnosing and quantifying co-infection by two globally distributed fungal pathogens of wheat. Front. *Plant Sci*. 9: 1086.

Aslam, S., Tahir, A., Aslam, M. F., Alam, M. W., Shedayi, A. A. and Sadia, S. (2017). Recent advances in molecular techniques for the identification of phytopathogenic fungi–a mini review. *Journal of Plant Interactions*, 12(1): 493-504.

Bhat, R. G. and Browne, G. T. (2010). Specific detection of *Phytophthora cactorum* in diseased strawberry plants using nested polymerase chain reaction. *Plant Pathol*. 59: 121–129.

Bilska, K., Kulik, T., Ostrowska-Kołodziejczak, A., Busko, M., Pasquali, M. and Beyer, M. (2018). Development of a highly sensitive FcMito qPCR assay for the quantification of the toxigenic fungal plant pathogen *Fusarium culmorum*. *Toxins* 10 (5): 211.

Brewer, M. T., Turner, A. N., Brannen, P. M., Cline, W. O., and Richardson, E. A. (2014). Carnielli-Queiroz, L., Fernandes, P. M. B., Fernandes, A. A. R. and Ventura, J. A. (2019). A rapid and reliable method for molecular detection of

Fusarium guttiforme, the Etiological Agent of Pineapple Fusariosis. *Braz. Arch. Biol. Technol*. 62, e19180591. Control, pp. 135–142.

Das, A., Sharma, N. and Prasad, M. (2019). CRISPR/Cas9: a novel weapon in the arsenal to combat plant diseases. *Frontiers in Plant Science*, 9: 2008.

Depotter, J. R. L., Rodriguez-Moreno, L., Thomma, B. P. H. A. and Wood, T. A. (2017). The Emerging British *Verticillium longisporum* Population consists of aggressive brassica pathogens. *Phytopathology*.107 (11): 1399–1405.

Doyle, V. P., Tonry, H. T., Amsden, B., Beale, J., Dixon, E. and Li, H. (2019). First report

Exobasidium maculosum, a new species causing leaf and fruit spots on blueberry in the southeastern USA and its relationship with other *Exobasidium* spp. parasitic to blueberry and cranberry. *Mycologia*.106 (3): 415–423.

Faganello, F. D. S., Filho, R. C., Dias, V. D., Morello, R. M. S.C. and DaCunha, M. G. (2017). Molecular diagnosis of *Guignardia citricarpa* in asymptomatic sweet orange tissue. *Rev. Bras. Frutic*. 39 (4): e–518.

Faria, C. B., Abe, C. A., da Silva, C. N., Tessmann, D. J. and Barbosa-Tessmann, I. P. (2012). New PCR assays for the identification of *Fusarium verticillioides*, *Fusarium subglutinans,* and other species of the *Gibberella fujikuroi* complex. *Int. J. Mol. Sci.* 13 (1): 115–132.

Gautam, A. K., and Kumar, S. (2020). Techniques for the detection, identification, and diagnosis of agricultural pathogens and diseases. In *Natural remedies for pest, disease and weed control* (pp. 135-142). Academic Press.

Halder, K., Chaudhuri, A., Abdin, M. Z., Majee, M. and Datta, A. (2022). RNA interference for improving disease resistance in plants and its relevance in this clustered regularly interspaced short palindromic repeats-dominated era in terms of dsrna-based biopesticides. *Frontiers in Plant Science*, 13: 885128.

Havis, N. D., Gorniak, K., Carmona, M. A., Formento, A. N., Luque, A. G. and Scandiani, M. M. (2014). First molecular detection of Ramularia leaf spot (*Ramularia collocygni*) in seeds and leaves of barley in Argentina. *Plant Dis*. 98 (2): 277–277.

Hayden R, Qian X, Procop G, Roberts G and Lloyd R. (2002). In situ hybridization for the identification of filamentous fungi in tissue section. *Diagn Mol Pathol*. 11(2):119–126.

https://en.wikipedia.org/wiki/Polymerase chain reaction.

Krishnamurthy PK and Francis R.A. (2012). A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodivers Conserv*. 21 (8):1901–1919.

Li D. Z., Gao L. M., Li H. T., Wang H., Ge X. J., Liu J. Q., Chen Z. D., Zhou S. L., Chen S. L., Yang J. B. (2011). Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proc Natl Acad Sci*. 108 (49):19641–19646.

Ling, K. S., Wechter, W. P., Somai, B. M., Walcott, R. R. and Keinath, A. P. (2010). An improved real-time PCR system for broad-spectrum detection of *Didymella bryoniae*, the causal agent of gummy stem blight of cucurbits. *Seed Sci. Technol*. 38 (3): 692–703.

Mancini, V., Murolo, S. and Romanazzi, G. (2016). Diagnostic methods for detecting fungal pathogens on vegetable seeds. *Plant Pathol*. 65: 691–703.

Martinelli, F., Scalenghe, R., Davino, S., Panno, S., Scuderi, G., Ruisi, P. and Dandekar, A. M. (2015). Advanced methods of plant disease detection. A review. *Agronomy for Sustainable Development*, *35*: 1-25.

McCartney H. A., Foster S. J., Fraaije B. A. and Ward E. (2003). Molecular diagnostics for fungal plant pathogens. *Pest Manag Sci*. 59(2):129–142.

Meghvansi, M. K., Khan, M. H., Gupta, R. and Veer, V. (2013). Identification of a new species of Cercospora causing leaf spot disease in *Capsicum assamicum* in northeastern India. *Res. Microbiol*. 164 (9): 894–902.

Michalecka, M., Bryk, H., Poniatowska, A. and Puławska, J. (2016). Identification of *Neofabraea* species causing bull's eye rot of apple in Poland and their direct detection in apple fruit using multiplex PCR. *Plant Pathol*. 65: 643–654.

Moffat, C. S., See, P. T. and Oliver, R. P. (2015). Leaf yellowing of the wheat cultivar Mace in the absence of yellow spot disease. *Australas. Plant Pathol*. 44: 161–166.

Ndayihanzamaso, P., Karangwa, P., Mostert, D., Mahuku, G., Blomme, G.and Beed, F. (2020). The development of a multiplex PCR assay for the detection of *Fusarium oxysporum* f. sp. *cubense* lineage VI strains in East and Central Africa. *Eur. J. Plant Pathol*. 158: 495–509.

Ndayihanzamaso, P., Karangwa, P., Mostert, D., Mahuku, G., Blomme, G., Beed, F. and Viljoen, A. (2020). The development of a multiplex PCR assay for the detection of *Fusarium oxysporum* f. sp. *cubense* lineage VI strains in East and Central Africa. *European Journal of Plant Pathology*, 158: 495-509.

Okawa, K. (2015). Market and trade impacts of food loss and waste reduction. *In: OECD Food, Agriculture and Fisheries Papers,* No. 75. OECD Publishing, Paris.

Patel, R., Mitra, B., Vinchurkar, M., Adami, A., Patkar, R., Giacomozzi, F. and Baghini, M. S. (2022). A review of recent advances in plant-pathogen detection systems. *Heliyon*.

Paul, N. C., Park, S. W., Liu, H., Choi, S., Ma, J., MacCready, J. S. and Sang, H. (2021). Plant and fungal genome editing to enhance plant disease resistance using the CRISPR/Cas9 system. *Frontiers in Plant Science*, 12: 700925.

Pépin, N., Punja, Z. K. and Joly, D. L. (2018). Occurrence of powdery mildew caused by plant and fungal genome editing to enhance plant disease resistance using the CRISPR/Cas9 system. *Front. Plant Sci.* 12:70092.

Prasannath, K., Galea, V. J. and Akinsanmi, O. A. (2020). Characterisation of leaf spots caused by *Neopestalotiopsis clavispora* and *Colletotrichum siamense* in macadamia in Australia. *Eur. J. Plant Pathol.* 156: 1219–1225.

Qi M and Yang Y. (2002). Quantification of *Magnaporthe grisea* during infection of rice plants using real-time polymerase chain reaction and northern blot/ phosphoimaging analyses. *Phytopathology*. 92 (8):870–876.

Raj, M., Jeeva, M. L., Nath, V. S., Sankar, S., Vidhyadharan, P., Archana, P. V. and Hegde, V. (2013). A highly sensitive nested-PCR method using a single closed tube for the detection of *Colletotrichum gloeosporioides* causing greater yam anthracnose. *Journal of Root Crops*, 39(2): 163-167.

Sankaran, S., Mishra, A., Ehsani, R. and Davis, C. (2010). A review of advanced techniques for detecting plant diseases. *Comput. Electron. Agric*. 72 (1): 1–13.

Sato T., Takayanagi A., Nagao K., Tomatsu N., Fukui T., Kawaguchi M., Kudoh J., Amagai M., Yamamoto N. and Shimizu N. 2010. Simple PCR based DNA microarray system to identify human pathogenic fungi in skin. *J Clin Microbiol*. 48(7):2357–2364.

Schoch C. L., Seifert K. A., Huhndorf S., Robert V., Spouge J. L., Levesque C. A., Chen W., Bolchacova E., Voigt K. and Crous P., W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc Natl Acad Sci U S A*. 109(16): 6241–6246.

Sikdar, P., Okubara, P., Mazzola, M. and Xiao, C. L. (2014). Development of PCR assays for diagnosis and detection of the pathogens *Phacidiopycnis washingtonensis* and *Sphaeropsis pyriputrescens* in apple fruit. *Plant Dis*. 98 (2): 241–246

Singh A. and Kumar N. (2013). A review on DNA microarray technology. *Int J Curr Res Rev*. 5(22):01–05.

Sun J, Li X, Zeng H, Xie Z, Lu C, Xi L and De Hoog GS. (2010). Development and evaluation of loop-mediated isothermal amplification (LAMP) for the rapid diagnosis of *Penicillium marneffei* in archived tissue samples. *FEMS Immunol Med Microbiol*. 58(3):381–388.

Sun, G., Liu, J., Li, G., Zhang, X., Chen, T. and Chen, J. (2015). Quick and accurate detection and quantification of *Magnaporthe oryzae* in rice using realtime quantitative polymerase chain reaction. *Plant Dis*. 99: 219–224.

Wang, X., Tang, C., Chen, J., Buchenauer, H., Zhao, J., Han, Q. and Kang, Z. (2009). Detection of *Puccinia striiformis* in latently infected wheat leaves by nested polymerase chain reaction. *Journal of phytopathology*, 157(7-8): 490-493.

Xu J. (2016). Fungal DNA barcoding. *Genome*. 59(11):913–932.

Yang, X., Hameed, U., Zhang, A. F., Zang, H. Y., Gu, C. Y. and Chen, Y. (2017). Development of a nested-PCR assay for the rapid detection of *Pilidiella granati* in pomegranate fruit. *Sci. Rep*. 7: 40954.

Yin, K. and Qiu, J. L. (2019). Genome editing for plant disease resistance: applications and perspectives. *Philosophical Transactions of the Royal Society B*. 374(1767): 20180322.

Zhang N, McCarthy ML and Smart CD. (2008). A macroarray system for the detection of fungal and oomycete pathogens of solanaceous crops. *Plant Dis*. 92(6):953–960.

Zhao, Y. Q., Wu, Y. H., Zhao, X. X., An, M. N. and Chen, J. G. (2014). Study on the Taqman real–time PCR to the detection and quantification of *Rhizoctonia solani* AG–3 of tobacco target spot. *Adv. Mater. Res*. 1010–1012: 80–83.

18 General Characteristics and Structures of Fungi

Dr. Satish Sharma[1], Dr. S.K Arsia[2], Dr. Arvinder Kaur[3] and Dr. Ashish Kumar Pandey[4]

[1]Assistant Professor (Contractual basis) , Department of Plant Pathology,RVSKVV, B.M. College of Agriculture ,Khandwa (M.P)
[2]Assistant Professor & Head , Department of Plant Pathology, RVSKVV, B.M. College of Agriculture ,Khandwa (M.P)
[3]Senior Scientist Plant Protection, RVSKVV, at Krishi Vigyan Kendra, Gwalior
[4]Subject Matter Specialist (Plant Protection) DRi LBS Krishi Vigyan Kendra, Gonda (U.P)

Abstract

Fungi are eukaryotic, spore bearing, achlorophyllous, heterotrophic organisms that generally reproduce sexually and asexually and whose filaments are branched somatic structures are typically surrounded by cell walls containing chitin or cellulose or both with many organic molecules and exhibiting absorptive nutrition to obtain nourishment i. e., for nutrition to resist or tolerate in unfavorable conditions for their survival i.e., over wintering, over summering for reproduction.

Keywords: Fungi characteristics, somatic structures and fungal nutrition**.**

Introduction

Mycology (Mycetology- Greek grammer): It is the Science which deals with study of fungi. Term Mycology derived from 2 Greek words. Mykes= mushroom / fungus, logos= discourse or study. The term Fungus is derived from a Latin word fungor = to flourish. Study of fungi started with study of mushrooms because of their macroscopic size and brilliant color. Mushrooms attracted the attention of ancient people,and they started studying them out of curiosity. Fungi are eukaryotic, spore

bearing, achlorophyllous. Pathogen is an entity usually a micro organism that can incite disease in susceptible plants. It is also referred to as incitant, causal agent or causal organism. Its branched somatic structures are typically surrounded by cell walls containing chitin or cellulose or both with many organic molecules and exhibiting absorptive nutrition. Fungal cells are typically eukaryotic and lack chloroplasts. Cell is bounded by cell wall, which provides rigidity and shape to the cell is the outermost membrane of cell consisting of more than one layer with fibrous structure and made up of chitin or cellulose or both.

Somatic structures

Thallus/ Soma Commonly called as vegetative body or fungal body. A thallus(pl. thalli) is a simple, entire body of the fungus devoid of chlorophyll with no differentiation into stem, roots and leaves lacking vascular system.

Hypha (hypha=web) (pl. hyphae) : Hypha is a thin, transparent, tubular filament filled with protoplasm.It is the unit of a filamentous thallus and grows by apical elongation.

Mycelium(pl. mycelia): A net work of hyphae (aggregation of hyphae) constituting the filamentous thallus of a fungus.It may be colourless i.e., hyaline or coloured due to presence of pigments in cell wall.The mycelium may be ectophytic or endophytic.

Types of fungal thalli:

1.Plasmodium (plasma = moulded body): It is a naked,multinucleate mass of protoplasm moving and feeding in amoeboid fashion . Eg. *Plasmodiophora brassicae.*

2.Unicellular thallus:consisting of a single cell. Eg.Chytrids, *Synchytrium*

3.Multi cellular or filamentous thallus: Majority of fungi i.e., a true fungi are filamentous, consisting of a number of branched, thread like filaments called hyphae. Eg.Many fungi,*Alternaria.*

Fungi based on reproductive structures:

Holocarpic (holos=whole+karpos=fruit): If the thallus is entirely converted into one or more reproductive structures, such thallus is called holocarpic thallus. Eg.*Synchytrium*

Eucarpic(Eu=good+karpos=fruit): If the thallus is differentiated into a vegetative part which absorbs nutrients and a reproductive part which forms reproductive structures, such thallus is called eucarpic thallus**.** Eg.*Pythium*

Ectophytic fungus: If the fungal thallus is present on the surface of the host plant, it is called ectophytic.Eg. *Oidium* .

Endophytic fungus: If the fungus penetrates into the host cell / present inside the host, it is called endophytic.Eg. *Puccinia*. Endophytic fungus may be **intercellular** (hypha grows in between the cells), or **intra cellular** (hypha penetrates into host cell).Eg.Ustilago, or **vascular** (xylem vessels) Eg. *Fusarium oxysporum.* Inter cellular hyphae produce special organs called haustoria which penetrate the host cell and absorb food. These are absent in intracellular hyphae. Endophytic intra cellular mycelium absorbs food directly from protoplasm without any specialized structures. In ectophytic mycelium, haustoria are produced in epidermal cells. **Septation in Fungi** :(septum=hedge/partition) (pl.septa). Some fungal hyphae are provided with partitions or cross walls which divide the fungus into a number of compartments / cells. These cross walls are called septa.

Aseptate hypha/coenocytic hypha: (Koinos=common,kytos=hollow vessel) A hypha with out septa is called aseptate /non-septate/ coenocytic hypha wherein the nuclei are embedded in cytoplasm.Eg. lower fungi like Oomycetes and Zygomycetes.

Septate hypha: A hypha with septa or cross walls is called septate hypha. Eg.common in higher fungi like Ascomycotina, Basidiomycotina and Deuteromycotina

General types of septa

1.Based on formation:

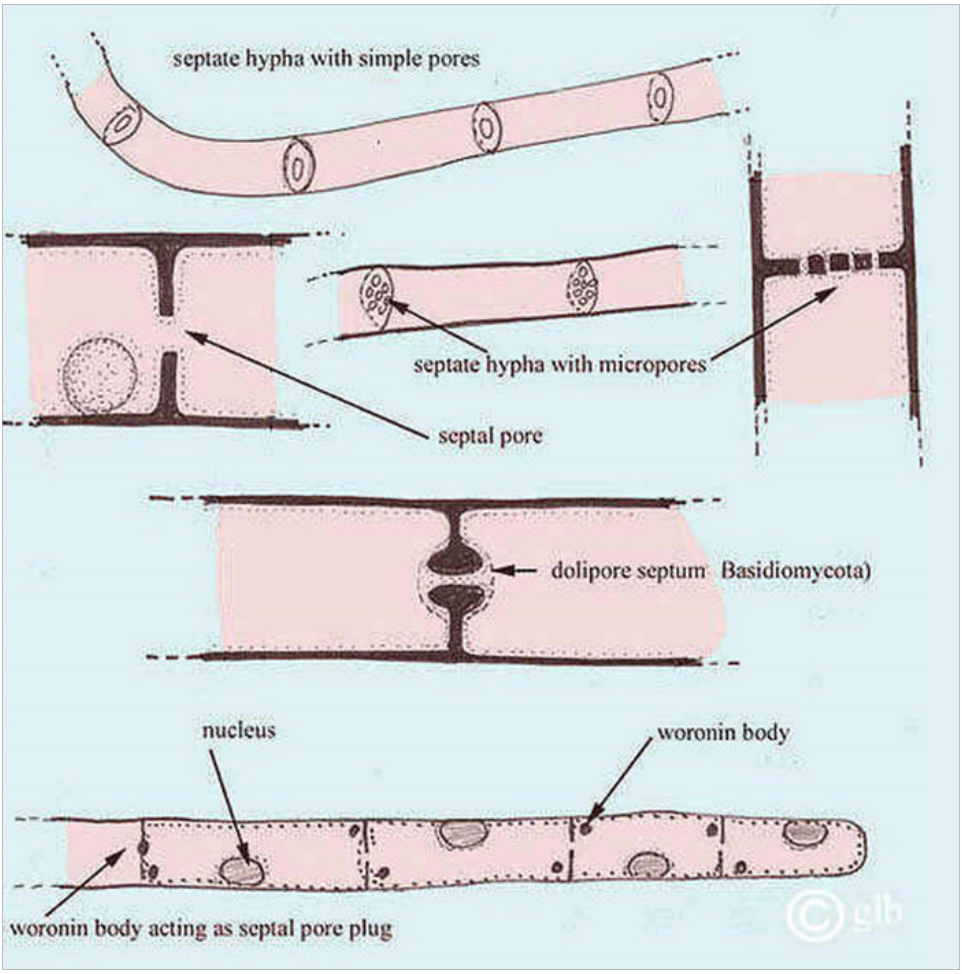
a) Primary septa: These are formed in direct association with nuclear division (mitotic or meiotic) and are laid down between daughter nuclei separating the nuclei /cells. Eg. Higher fungi like Ascomycotina and Basidiomycotina.

b) Adventitious septa: These are formed independent of nuclear division and these are produced to delimit the reproductive structures. Eg. lower fungi like Oomycetes and Zygomycetes in which septa are produced below gametangia (sex organs) which separate them from rest of the cells.

2. Based on construction:

a) Simple septa: It is most common which is a plate like, with or without perforation.

b) Complex septa: A septum with a central pore surrounded by a barrel shaped swelling of the septal wall and covered on both sides by a perforated membrane termed the septal pore cap or parenthosome. Eg. Dolipore septum in Basidiomycotina.



3.Based on perforation:

a) Complete septa: A Septum is a solid plate without any pore or perforations. Eg. Adventitious septa in lower fungi.

b) Incomplete septa: A septum with a central pore.

Fungal tissues: Plectenchyma :(plekein=to weave+enchyma=infusion**)** Fungal tissues are called plectenchyma i.e., mycelium becomes organized into loosely or compactly woven tissue. This tissue compose various types of vegetative and reproductive structures.

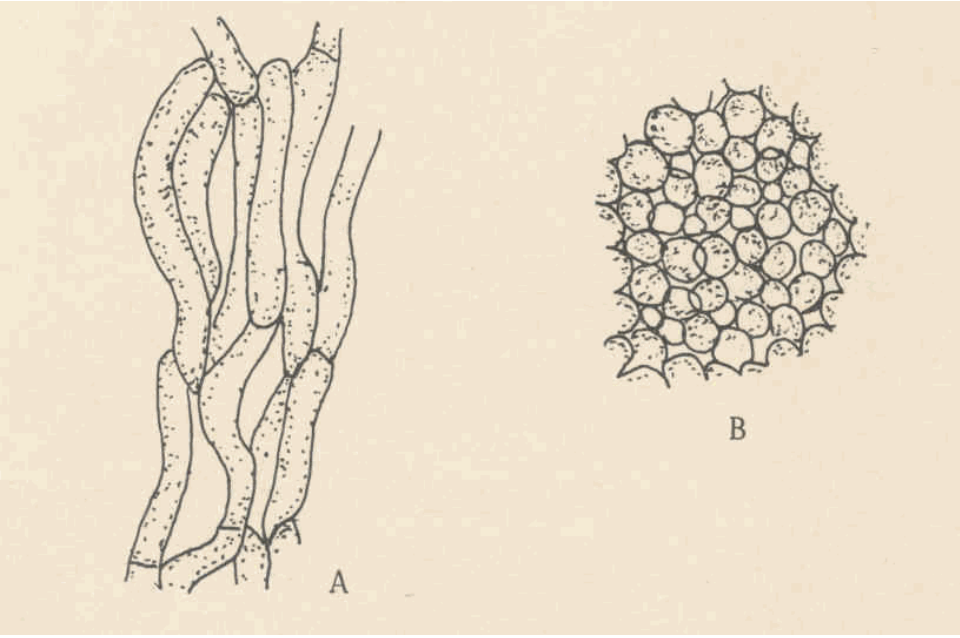
Types of plectenchyma:

1.Prosenchyma: It is a loosely woven tissue. The component hyphae retain their

individuality which can be easily distinguishable as hyphae and lie parallel to one another.Eg. Trauma in *Agaricus.*

2. Pseudoparenchyma:It is compactly woven tissue. It consists of closely packed cells which are isodiametric or oval in shape resembling parenchymatous cells of plants and hence the name. The component hyphae loose their individuality and are not distinguishable as hyphae. Eg. Sclerotial bodies of *Sclerotium* and rhizomorph of *Armillariella.*

A.Prosenchyma B.Pseudoparenchyma



Purpose:

1. To obtain nourishment i. e., for nutrition.

2. To resist or tolerate unfavourable conditions for their survival i.e., over wintering, Over summering.

3. for reproduction.

1. Rhizomorphs: (rhiza=root, morph=shape) Thick strands of somatic hyphae in which the hyphae loose their individuality and form complex tissues that are resistant to adverse conditions and remain dormant until favourable conditions return. The structure of growing tip of rhizomorphs res emble that of a root tip, hence the name rhizomorph. Eg. *Armillariella mellea.*

2. Sclerotium: (skleron=hard) pl.sclerotia: It is a hard, round (looks like mustard seed)/ cylindrical or elongated (*Claviceps*) dark coloured (black or brown)resting body formed due to aggregation of mycelium, the component hyphae loose their individuality , resistant to unfavourable conditions and remain dormant for a longer period of time and germinate on the return of favourable conditions. Eg. *Sclerotium, Rhizoctonia* .

3. Stroma: (stroma=mattress) pl.stromata. It is a compact somatic structure looks like a attress or a cushion on which or in which fructifications (spores or fruiting bodies) are usually formed.

a. Sub stomatal stroma: cushion like structure formed below epidermis in sub stomatal region from which sporophores are produced. Eg. *Cercospora personata.*

b. Perithecial stroma: When reproductive bodies like perithecia of some fungi are embedded characteristically throughout periphery of stroma, such stroma are called perithecial stroma. Eg. *Claviceps, Xylaria.*

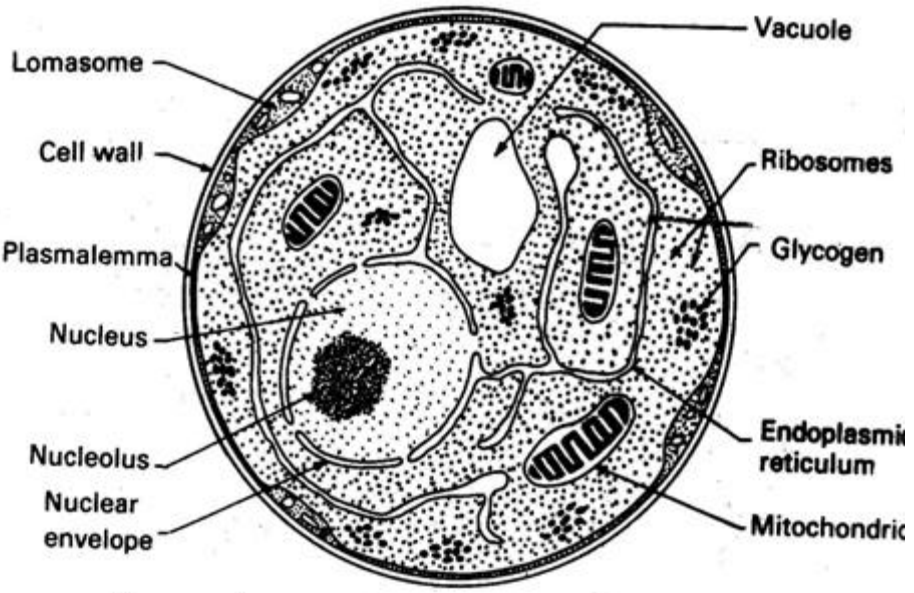
4. Haustorium: (hauster=drinker) pl.haustoria.It is a outgrowth of somatic hyphae regarded as special absorbing organ produced on certain hyphae by parasitic fungi for obtaining nourishment by piercing into living cells of host. They may be knob like(*Albugo),* elongated *(Erysiphe, Uncinula),* finger like (*Peronospora)*.

5.Rhizoids: (rhiza=root, oeides=like) These are slender root like branched structures found in the substratum produced by some fungi which are useful for anchoring the thallus to substratum and for obtaining nourishment from the substrate.Eg. *Rhizopus stolonifer.*

6. Appresorium: (apprimere=to press against) pl.appressoria A flattened tip of hyphae or germ tube acting as pressing organ by attaching to the host surface and gives rise to a minute infection peg which usually grows and penetrates the epidermal cells of the host. Eg. *Puccinia, Colletotrichum, Erysiphe.*

Fungal cell :

Fungal cells are typically eukaryotic and lack chloroplasts. Cell is bounded by cell wall, which provides rigidity and shape to the cell is the outermost membrane of cell consisting of more than one layer with fibrous structure and made up of chitin or cellulose or both.



Diagrammatic representation of the ultrastructure of a cross section of a typical fungus cell.

The layer surrounding the cytoplasm is called cytoplasmic membrane or plasmalemma. Protoplasm contains a true nucleus surrounded by two layered membrane with nucleolus, cytoplasm and other inclusions.

Endoplasmic reticulum is not well developed, and it may be rough atudded with ribosomes or smooth with out ribosomes.

Vacuoles in which metabolic products are accumulated are bounded by a membrane called tonoplast . Ribosomes are protenaceous bodies scattered all over cytoplasm, play a role in protein synthesis. Mitochondria are the sites of respiratory activities. Lomasomes are the swollen membranous structures of plasmalemma. Cytoplasm also contains fat particles, calcium oxalate crystals, resins, glycogen.

Fungal nutrition:

Fungi are heterotrophic with holophytic nutrition(absorptive type). The essential elements for fungi are, C, H, O, N, P, K, S, Zn, Fe, Mg, Mn, Mo, Cu and Ca. Reserve food material in the c ell may be either fat or carbohydrates. Fats may be present in the form of oil drops and carbohydrates in the form of glycogen or sugars. Starch is never present in the fungal cell.

Groups of fungi based on mode of nutrition:

1. Saprophytes: (sapros=rotten, phytos=plant) Organisms which obtain nutrition on from dead organic matter either completely or for a part of their life. A large number of fungi fall under this category. Eg. *Saprolegnia, Rhizopus, Mucor, Alternaria.*

***a.* Obligate saprophytes:** (obligare =to bind it self) Organisms which can never grow on living organisms or can never obtain their food from living source. They get their food only from dead organic matter. Eg. *Mucor, Agaricus* .

b. Facultative parasite: (facultas=ability) Organisms which are usually

saprophytic but have ability to become as parasites. Eg. *Pythium aphanidermatum, Fusarium solani, Rhizoctonia solani.*

2.Parasites: Organisms which live within or out side another organisms for their nutrition either completely or for a part of their life .

Pathogen : If a parasite damages the host then they are called as pathogens..

All pathogens are not parasites and all parasites need not be pathogens .

a. Obligate parasites: (Organisms which obtain food only from living organisms (living protoplasm) and can never derive their food from dead organic matter or artificial medium. Eg. *Puccinia graminis* , *Plasmopara viticola* .

b. Facultative saprophytes: Organisms which are usually parasites but have ability to become saprophytes .Eg. *Ustilago maydis*

Classification of fungi based on sex :

1. Monoecious fungi / hermaphroditic fungi: (mono=single,oikos=home) The fungi which produce distinguishable male and female sex organs on the same thallus, which may or may not be compatible are called monoecious/ hermaphroditic fungi. Eg. *Pythium aphanidermatum*.

2. Dioecious fungi: (di=two, oikos=home) The fungi which produce distinguishable male and female sex organs on two different thalli ie., there will be separate male and female thalli . Eg. *Phytophthora infestans*

Classification of fungi based on compatibility

Homothallic fungi: Fungi in which both sexes occur on same thallus, which can reproduce sexually by it self with out the aid of another thallus ie., self compatible / self fertile are called homothallic fungi.

Eg. *Pythium aphanidermatum.*

Heterothallic fungi: A fungal species consisting of self sterile (self incompatible)

thallus requiring the union of two compatible thalli for sexual reproduction, regardless of the possible presence of both male and female organs on the same thallus. Heterothallic fungi are dioecious

Eg. *Phytophthora infestans.*

Different types of sexual spores : Sexual spores are formed after meiosis,hence also called meiospores. 1.Oospores 2.Zygospores 3.Ascospores 4.Basidiospores

1.**Oospore:** A thick walled sexual resting spore produced by the union of two morphologically different gametangia. Eg.*Pythium, Phytophthora* , members of class Oomycetes.

2. Zygospore: A thick walled sexual resting spore produced by the fusion of two morphologically similar gametangia. Eg.*Rhizopus* , members of sub-division Zygomycotina

3. Ascospore:Sexual spore produced in a specialized sac like like structure known as ascus. Generally 8 ascospores are formed. Eg.*Erysiphe,*members of sub-division Ascomycotina.

4.**Basidiospore:**Sexual spore produced on a club shaped structure known as basidium.Generally 4 basidiospores are formed.Eg.*Puccinia,* members of sub-division Basidiomycotina.

Various Life cycle patterns displayed by fungi:

1. Haplobiontic life cycle 2. Diplobiontic life cycle

1. **Haplobiontic life cycle**: If there is only one free living thallus, which is haploid or diploid in life cycle of a fungus, it is called as haplobiontic life cycle. (long haploid somatic phase and short diploid phase confined to zygote cell, which undergoes meiosis immediately after karyogamy and develop ascospores) Eg. *Schizosaccharomyces octosporus.*

2. **Diplobiontic life cycle:** If haploid thallus alternates with a diploid thallus, the life cycle is called diplobiontic life cycle, which has a long diploid somatic phase and a very short haplo Id phase. Eg. *Saccharomyces ludwigii*

Phases in Sexual reproduction:. There are 3 phases in sexual reproduction.

1. **Plasmogamy**: union of two protoplasts takes place.As a result of it the two nuclei come together within the same cell.

2. **Karyogamy**: union of 2 sexually compatible nuclei brought together by plasmogamy to form a diploid nucleus (2n) i.e., zygote.

3. **Meiosis**: This is reduction division . The number of chromosomes is reduced to

haploid (n) i.e., diploid nucleus results into haploid nucleus..

In lower fungi (Phycomycetes -Mastigomycotina and Zygomycotina) plasmogamy, karyogamy and meiosis occurs at regular intervals / sequence i.e.,karyogamy follows immediately after plasmogamy. In higher fungi (Ascomycotina, Basidiomycotina), karyogamy is delayed, as a result the nuclei remain in pairs (dikaryotic phase- n+ n condition), which may be brief or prolonged.

Dikaryon : A pair of genetically different nuclei, lying side by side with out fusion for a considerable period of time is called dikaryon.A cell containing dikaryon is called **dikaryotic cell**. And the process is known as **dikaryogamy**

Conclusion

Plant diseases caused by micro organisms are of param ount importance to humans because they damage plants and plant products on which human depend for food, clothing, furniture and housing. for nutrition to resist or tolerate in unfavorable conditions for their survival i.e., over wintering, over summering for reproduction. A net work of hyphae (aggregation of hyphae) constituting the filamentous thallus of a fungus.It may be colourless i.e., hyaline or coloured due to presence of pigments in cell wall.The mycelium may be ectophytic or endophytic. The essential elements for fungi are, C, H, O, N, P, K, S, Zn, Fe, Mg, Mn, Mo, Cu and Ca.Reserve food material in the c ell may be either fat or carbohydrates. Fats may be present in the form of oil drops and carbohydrates in the form of glycogen or sugars. Starch is never present in the fungal cell.

References

Alexopoulos, C.J., Mims C.W. and Blackwell M. 1996. *Introductory Mycology.* Wiley Eastern Ltd, New York.

Mandahar, C.L. 1987. *Introduction to Plant Viruses.* Chand and Co Pvt Ltd, New Delhi.

Mehrotra, R.S. and Aneja, K.R. 1990. *An Introduction to Mycology.* New Age International (P) Ltd, New Delhi.

Singh, R.S. 1982. *Plant Pathogens - The Fungi. Oxford* & IBM Publishing Co. Pvt. Ltd., New Delhi.

Singh, R.S. 1989. *Plant Pathogens - The Prokaryotes.* Oxford & IBM Publishing Co. Pvt. Ltd., New Delhi.

19 Causal Organisms for Plant Diseases and its Symptoms

[1]Dr Ashish kumar Pandey, [2]Dr. Satish Sharma, [3]Sudhanshu and [4]Pushpendra Singh Gurjar

[1]Subject Matter Specialist (Plant Protection), DRI LBS Krishi Vigyan Kendra, Gonda (U.P.) India
[2]Assistant Professor (Contractual basis), Department of Plant Pathology, B. M. College of Agriculture Khandwa
[3]Subject Matter Specialist (Agricultural Extension), DRI LBS Krishi Vigyan Kendra, Gonda (U.P.) India
[4]Subject Matter Specialist (Horticulture), DRI LBS Krishi Vigyan Kendra, Gonda (U.P.) India

Abstract

Plant pathology is a very important area of plant sciences because of its key role in sustainability of crop production and productivity. The chapter Fundamentals of Plant Pathology has been written to meet the needs of students for plant pathology courses at various levels of graduate and postgraduate studies. For understanding plant pathology, it is essential to know the basic aspects like pathogens, disease development, plant defiance mechanisms against pathogens and disease management. This chapter all the basic aspects of plant pathology viz., importance of plant diseases; scope and objectives of plant pathology; history of plant pathology; terms and concepts in plant pathology; pathogenesis; disease development; plant pathogens: Fungi, bacteria, fastidious vascular bacteria, phytoplasmas, spiroplasmas, virus, viroid, algae, protozoa, phanerogamic parasites and nematodes; classification of diseases and pathogens; symptomatology of biotic and abiotic stresses, growth and reproduction of plant pathogens; liberation, dispersal and survival of plant pathogens; types of parasitism

and variability in plant pathogens, pathogenesis, role of enzymes, toxins and growth regulators in disease development; defence mechanism in plants; epidemiology and disease forecasting; principles and methods of plant disease management; nature, chemical combination, classification, mode of action and formulations of fungicides and antibiotics against pathogens. This chapter is very useful for students, teachers and researchers.

Keywords: Causal Organisms of Plant diseases, Symptoms, Disease cycle and Control measures.

Introduction

Fungi are a group of such plants which lack root system and chlorophyll. Hence, they lack thecapacity of mineral absorption and photosynthesis. They obtain their nutrition from other living or dead plants and animals. Most fungi are saprophytic, i.e., they obtain their nutrition from dead organic matter. There are certain fungi which obtain their nutrition from living cells. These are known as parasitic fungi. These fungi also affect the physiological processes of the host. The toxins produced by certain fungi cause morphological deformity in the host. Such effects of the parasite appear in the host plant in the form of disease symptoms. The late blight epidemics of the 1840s triggered the Irish potato famine, but the history of the potatoas a food crop is much older. The potato (*Solanum tuberosum*) originated in the highlands of the Andes in the Lake Titicaca area between Bolivia and Peru, where native people had selected hundreds of different cultivars for centuries. Most scientists agree that *Phytophtora infestans* originated in Mexico where both mating types of the fungus are commonplace. Where and how the plant and the pathogen first came together is not certain, but late blight epidemics seem to be described in the northeastern U.S. in about 1843 and Europe in 1845. Potato crops failed for a number of years during the cool and rainy "hungry '40s." Although poor people who were dependent on potatoes for food suffered in many areas, the disasterwas greatest in Ireland. One and one-half million people starved and a similar numberemigrated during the famine, resulting in a large Irish diaspora in many parts of North America. As with many famines, politics enhanced the suffering. Many Irish peasants grew cereal crops to pay their rent. Although the grain was harvested, it could not be eaten, and was exported to the English landlords throughout the famine. In the 1990s, many exhibits andgatherings in North America and Ireland commemorated the 150th anniversary of the famine.One reason that the early history of late blight is unclear is that the germ theory of diseasehad not yet been accepted. Many preliminary studies of various plant diseases had been conducted, but Anton de Bary's (the "father of plant pathology") conclusive studies finally convinced the scientific community that the

white sporulation of *P. infestans* on infected plants was the causal agent of the disease and not the result of spontaneous generation from the decaying vegetation. Thus, late blight signifies the official beginning of the science of plant pathology. These early studies also contributed to Louis Pasteur's germ theory which was proposed 15 years later. Red rot is one of the major constraints in the profitable cultivation of sugarcane in many states of India. Except Maharashtra, the disease has been recorded in all the states. This disease drastically retards the yield and considerably deteriorates the juice quantity and quality thus hitting both the cane growers and millers. Many good varieties have gone out of cultivation due to red rot. Loose smut of wheat is a common disease throughout the wheat-growing regions of the world. The mycelium remains dormant in the embryo, and developing kernels are replaced by black teliospores. No seeds develop in infected heads. The disease is spread by windblown teliospores. Cool, humid weather favors the development of this disease. Some of the important fungal diseases of plants are discussed in this chapter.

Late Blight of Potato

The late blight is one of the destructive diseases of potato. It is the most serious of all the potato diseases when conditions are favourable for its spread. The famous Irish famine of 1845-46 was due largely to the failure of potato crop due to late blight infection. In India, the disease was first introduced into the Nilgiri hills between 1870 and 1880 and very soon, it spread to Darjeeling in the Himalayan ranges. The first severe outbreaks of the disease were reported between 1912 and 1928 from Assam, Bengal and Bihar. In northern India, the disease was first reported from the plains of western U.P. Since then, the disease appears as a regular feature in the plains causing severe losses to the potato crop.

.Leaves, stems and tuber showing late blight symptoms

Symptoms

The symptoms of the disease can be seen on any part of the plant, viz., leaves,petioles, stems and tubers .On the leaves, the symptoms appear in the form of dark brown, oval or irregular water soaked areas. In the early stages, the symptoms develop at the tips or the margins of the old leaves. The infection spreads vigorously when temperature is low and atmosphere is humid, and soon appears in the form of blight. In case of severe infection,practically all the parts of the host become brown and then degenerate. After the tops have been blighted, the infection reaches to the underground tubers. In the infected tubers, the skin becomes slightly sunken and dark in colour. If conditions are humid, the cells of the tuber become soft and dark brown. This symptom is known as **wet rot**. In dry atmosphere, however, the potato pulp does not rot but its anterior part becomes black. This condition is called **dry rot.**

Morphology of the causal organism

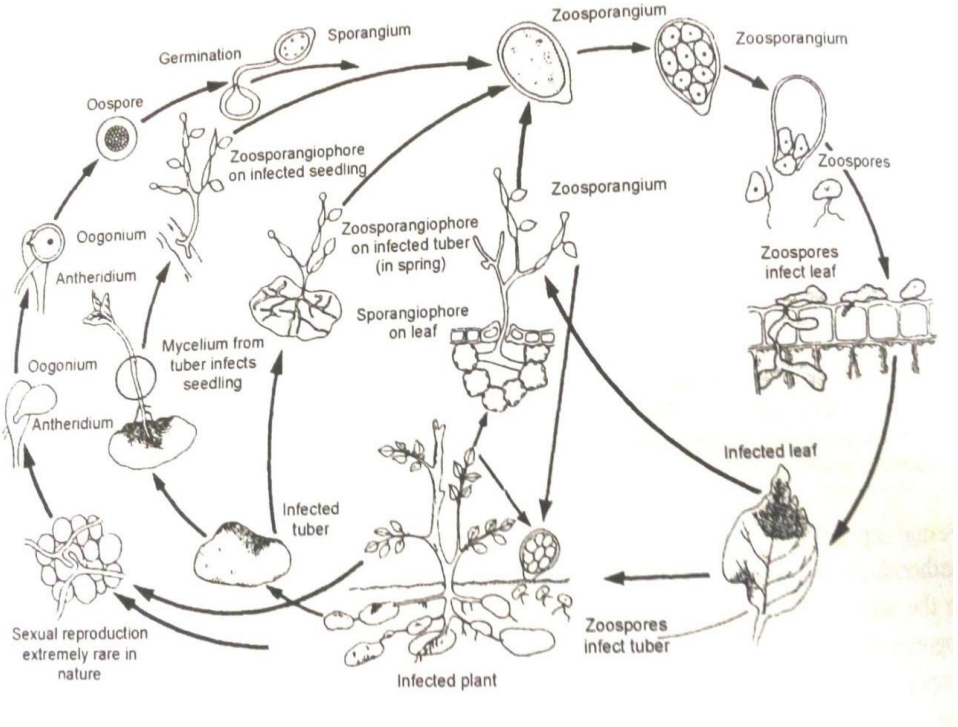
The disease is caused by *Phytophthora infestans*. The mycelium is endophytic consisting of hyaline, profusely branched coenocytic hyphae. The hyphae develop intercellularly and form haustoria. Ovoid or lemon-shaped sporangia are produced on sporangiophores. The sporangia are at first terminal but become lateral due to sympodial branching of the sporangiophore. The sporangium may germinate directly, forming a germ tube at the apex or its protoplasmic contents divide to form a number of biflagellate zoospores which emerge through the papilla. The method of germination is much governedby temperature; low temperature favours zoospores formation, whereas higher temperature is responsible for the germ tube development. Sexual reproduction is oogamous type. The antheridium is somewhat elongated and amphigynous, whereas the oogonium is pear-shaped to almost spherical, smooth and reddish brown in colour. The fusion of egg and male nucleus results in the formation of a diploid oospore. The oospore germinates by producing a germ tube and the tip of which tube ultimately develops in to a sporangium.

Disease cycle

The infected tubers are the main source of infection. Thedormant mycelium in the tubers becomes active and grows upward in the stem and sporulates on small shoots. Epiphytotics of the disease are likely to occur when unusually cool weather combined with abundant moisture prevails at the time the sporangia are being produced. The optimum temperature for the sporulation is 21°C. The sporangia germinate giving rise to zoospores at 12°C and by germ tube, 21°C. 100 percent relative humidity causes abundant production of sporangia. Sporangia are easily detached and disseminated

by rain or air. On reaching a suitable host, the sporangia germinate either by germ tubes or by zoospores depending upon the environmental conditions. The spores from the blighted leaves are washed down into the soil where they penetrate to different depths reaching the healthy tubers. Contact of healthy tubers with diseased leaves at the harvesting time is another source of tuber infection.

Several theories have been put forth to explain the yearly occurrence of late blight; but (i) persistence of mycelium in the affected tubers, and (ii) survival of fungus in the fruiting stageor as dormant mycelium in the tubers left in the field from the preceding crop, constituting the primary inoculum are the most accepted ones. In Indian climatic conditions, the possibility of survival of the pathogen through soil in any form is very remote. Thus, it seems the infected seed tubers are responsible for the spread of the disease.



***Phytophthorainfestans* causing late blight disease of Potato (Disease cycle)**

Control measures

The late blight can be effectively controlled by the following methods:

» Healthy seed tubers.

- » Field sanitation.
- » Delayed harvesting and sorting potatoes from a blighted field.
- » Tuber treatment before storage.
- » Storage in a cool, dry and well aerated store house.
- » Use of disease-resistant varieties, like Kufri red, Kufrineela, Kufrikundan, etc.
- » Foliar spray of fungicides like Bordeaux mixture, Dithane Z-78, Fytolan, Blitox, etc.
- » Good late blight management practices include disease prevention, sanitation, culturalpractices, field monitoring, an effective fungicide spray program and postharvest protection.

Sanitation, Cultural Practices and Field Monitoring

Plant disease-free seed. Inspect seed potatoes within 24 hours of delivery. Cut a sample of tubers and look for the reddish brown dry rot characteristic of late blight tuber rot.

Test the seed lots for late blight before planting. When purchasing seed, it is recommendedto have them tested for the absence of blighted seed by an authorized provincial service in your region.

Grade seed potatoes after being cut to remove any late blight infected tubers. Infected tubers can be a potential source of early infections in the field.

Frequently disinfect seed cutting equipment.

Immediately after cutting, treat seed with a recommended mancozeb-based seed piece fungicide.

Bury cull piles before crop emergence. Infected tubers in cull and rock dump piles are a major source of infection for the new crop. Buried tubers may germinate and grow. Rogue or treat volunteer plants with a herbicide. Slivers and pieces of potato remaining from cutting operations should also be buried.

Volunteer potato plants can be a source of infection. Any volunteer potato plants in a field should be removed by rouging or using herbicides. For non-seed fields where late blight is found, consider applying a sprout inhibitor to control volunteers in the following year.

Controlling late blight susceptible Solanacaeous weeds such as hairy nightshade, in potato as well as in non-potato crops is an important measure of controlling late blight incidence in potato.

Immediately report any suspected incidence of late blight to your extension specialist or to the nearest agricultural center. If late blight is identified, roguers and other workers should wear pants and boots which can be disinfected (e.g. Bleach solution diluted 1:9 with water; or other disinfectants) between different fields. Equipment should also be washed and disinfected before entering adjoining fields.

Construction of a deep hill may help restrict spores from washing down through the soil and infecting the developing tubers.

Weather conditions favorable for late blight development can be determined using late blight forecasting models that use relative humidity, rainfall and temperature data. Theweather data is converted into units called "severity values" for the purpose of predicting late blight outbreaks. Consult your extension specialist for information on late blight forecasts for your area.

Monitor your crop. Scout fields with special attention to low spots and along tree edges where moisture persists after rains or dews. Have a good look at stems and leaves for late blight symptoms. Stem infections will be diminished during dry periods but will be re- activated in humid weather.

When late blight is first identified, top kill or rogue an area twice the size of the infected area. All rogued infected plants should be put in plastic bags and then taken out of the field.

Rolling or rotobeating a crop before top killing would expose the soil and lower canopyto drying. Rolling also seals cracks in the soil and may reduce tuber infections.

Top kill at least 2 weeks prior to harvest to allow time for infected tubers to rot and to promote tuber maturity and thicker skins at harvest. Vines should be completely dead at harvest. 16. Late blight causing spores survive longer in wet soils. Harvest when the soil surface is dry or wind row the potatoes and allow the surface of tubers to dry before harvest.

Dig potential problem areas such as sprayer rows and low areas last and store these potatoes where they can be easily moved out in case of a problem.

Wet or bruised tubers are more likely to get infected with late blight. Skinned or cut and bruised areas are direct entry points for late blight and other diseases. However, wound is not always a requirement to occur an infection on wet tubers.

Grade out any obviously diseased potatoes before they are put into storage.

If late blight is seen on the foliage, there will also likely be tuber infections. Immediately following harvest, these tubers should be ventilated with a high volume

of air at low humidityuntil the surface of the potatoes is dry. This may lead to higher shrinkage than normal, but losses due to storage rots will be reduced.

Potato lots with 5% or more late blight infections (by weight) should be stored in the frontof the storage or in separate bins, so they can be easily removed in a high risk situation.

Postharvest treatment with fungicides containing phosphorous acid will protect healthy tubers from pink rot or late blight infections occurring at harvest. Ensure even coverage with the fungicide. Follow label rate and recommendations.

Fungicide Spray Programme:

A preventive spray program is always recommended. Effective control by fungicides requiresgood coverage of the foliage, proper rates and timing of applications. Generally, fungicides are most effective in the early stages of infections before symptoms appear. However, no fungicide can cure an established infection. Fungicides against late blight are essentially protectants and not particularly persistent. They must be used to protect plants as prophylacticsprays in routine programmes, in an overall strategy designed to prevent the disease infecting the crop.

Contact fungicides retain on the surface of the plant where these are applied and only protect the plant where the spray is deposited or subsequently re-distributed by moisture. Contact fungicides are not taken into the plant and therefore are vulnerable to erosion by wind, rain and degradation by sunlight. They do not protect new plant growth formed after the spray hasbeen applied. These fungicides have no effect against already established late blight infections.

Translaminar fungicides are absorbed by the leaves and show limited redistribution from upper sprayed surface to lower unsprayed surface. They are generally more rainfast than contact fungicides, but do not move within the plant to protect the new growth.

Systemic fungicides are absorbed into plant tissue and may offer some after-infection activity. Very few fungicides are truly systemic (i.e., move freely throughout the plant); however, some are upwardly systemic (i.e., move only upward in the plant through xylem tissue), and some are locally systemic (i.e., move into treated leaves and redistribute to some degree within the treated area of the plant.

RED ROT OF SUGARCANE

Red rot of sugarcane is a serious disease prevalent wherever sugarcane is grown in theworld. The disease was first described in Java in 1893 under the name red smut. In 1906, Butler reported the disease symptoms in several cane varieties in India and renamed it as red rot. Serious Epiphytotics of this disease have occurred in Northern India (U.P., and Bihar during 1939-40 and 1946-47). However, localized epidemics occur almost every year.

Symptoms

The disease appears on all aerial parts of the plant. The early symptoms of the disease are yellowing and drooping of leaves. The stems show little indication of the disease in early stages but as severity of the disease increases the cane splits lengthwise and subsequently red blotchesappear throughout the length of the cane. These blotches emit a peculiar smell of alcohol fermentation. The reddening appears mainly in the vascular region. Ultimately, the cane becomes dull in appearance, get rotten and shrinks at the internodes. Besides, the conspicuous symptoms also appear on the leaves. On the mid-ribs of leaves, infection originates as a dark reddish area which elongates rapidly, forming blood-red lesions with darkmargins. In later conditions, the centre of the lesions becomes straw coloured.

Yellowing and drooping of leaves and reddening of stems shows the Red rot disease inSugarcane

Morphology of the causal organism

The red rot of sugarcane is caused by *Colletotricum falcatum*. The perfect stage of this fungus has been described in Ascomycetes as *Glomerella tucumanensis*. The mycelium is inter-or intracellular, profusely branched, septate and contains characteristic oil droplets. Inlater stages the hyphae closely interwine with one another and form small stromata under the host epidermis. The fungus reproduces asexually by the formation of conidia developed in acervuli. The conidiophores are usually aseptate, unbranched and are arranged compactly likepalisade tissue. Usually a single conidium develops at the tip of each conidiophore but occasionally the conidia are produced

in acrogenous chains. The conidia are hyaline, crescentor sickle-shaped. An oil drop is present in the centre of each conidium. The conidia germinateby producing 1 to 4 germ tubes and form the new mycelium.

Perithecia are globose and are produced on the various part of the host, and measure 150-300µm in diameter. Asci are numerous, hyaline, clavate and measure 50-60 × 7-10.5µm. Each ascus contains eight ascospores arranged biserially. Intermixed with asci are present numerous delicate paraphysis. New physiological races differing in their pathogenicity to sugarcane cultivars have been frequently reported from different parts of the world.

Disease cycle

Seed sets from diseased canes are the chief means of survival and annual occurrence of the disease. Once, the pathogen establishes, secondary spread occurs by conidia. Ratoon crops also serve as a source of perennation and inoculum multiplication. High humidity, water-logging conditions, lack of proper cultural operations and continuous cultivation of same variety year after year are the main factors responsible for the development of the disease.

Control measures

The following measures may be adopted for the control of red rot of sugarcane:

- Healthy seed selection.
- Cultural practices-ratooning results in the multiplication of the disease, hence it mustbe discouraged.
- Field sanitation and crop rotation.
- Use of resistant varieties like Co 846, 951, 975, 1007, 1148, 62101, 62399, S109, BO22, 22, COLK 7702, 7710, etc.
- Treatment of seed setts with organo-mercurials like aretan or agallol (0.25% suspension) helps in eradication of superficial inoculum. Besides, treatment of setts in 0.5% bavistin solution reduces the incidence of red rot from infected setts.

LOOSE SMUT OF WHEAT

Loose smut, a very serious disease of wheat, is world-wide in occurrence and is a serious problem in the humid and semi-humid wheat growing regions. In India, the disease occurs in all wheat growing areas, but its incidence is higher in the cooler and moist northern parts than in the south. According to an estimate, the disease causes about 40% loss in wheatyield every year.

Symptoms

It is very difficult to detect infected plants in the field until heading. At this time, infected heads emerge earlier than normal heads. The entire inflorescence is commonly affected and appears as a mass of olive-black spores, initially covered by a thin gray membrane .Once the membrane ruptures, the head appears powdery. Spores are dislodged, leaving only the rachis intact. In some cases remnants of glumes and awns may be present on the exposed rachis. Smutted headsare shorter than healthy heads due to a reduction in the length of the rachis and peduncle. All or a portion of the heads on an infected plant may exhibit these symptoms. While infected heads are shorter, the rest of the plant is slightly taller than healthy plants. Prior to heading affected plants have dark green erect leaves. Chlorotic streaks may also be visible on the leaves.

Loose smut of wheat: infected inflorescence

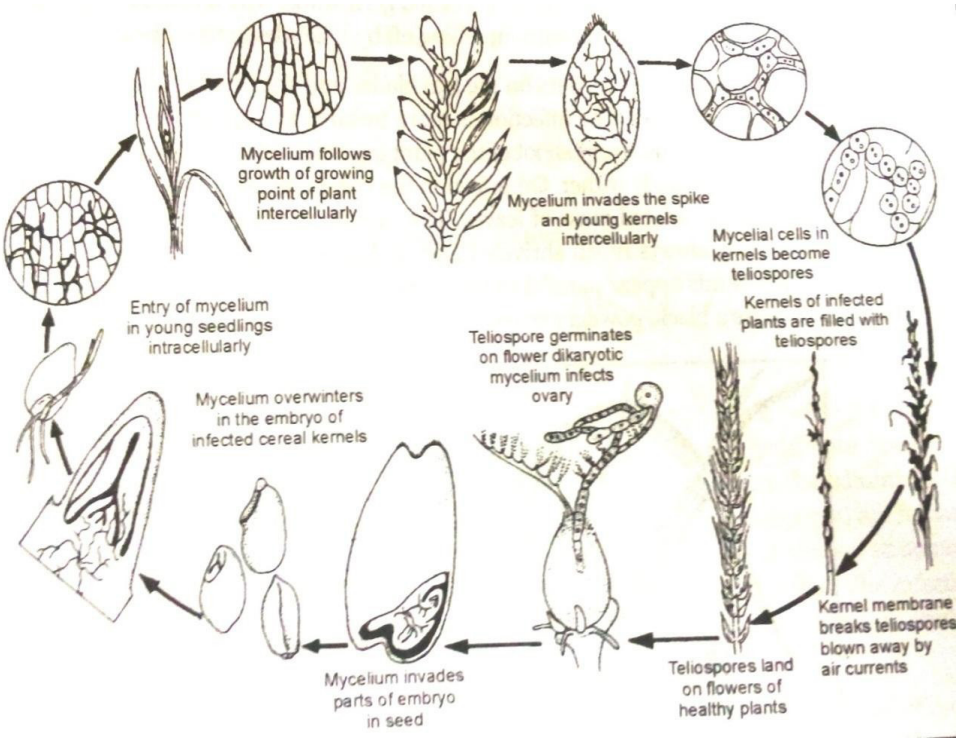
Morphology of the causal organism

The fungus responsible for the disease is *Ustilago nuda*var *tritici*. The mycelium of this fungus consists of multicelluar hyphae which are hyaline but turn brown at maturity. The primary mycelium is septate and uninucleate, whereas the secondary mycelium is branched, septate and bi-nucleate. In vegetative phase the secondary mycelium is profusely branched and spreads in intercellular spaces of the host tissues. The pathogen reproduces by the formation of chlamydospores and basidiospores. The hyphal cells are transformed into olivaceous brown, spherical and echinulate chlamydospores which germinate readily and produce basidia and basidiospores. The haploid basidiospores produce uninucleate primary mycelium on germination.

Disease cycle

Ears of infected plants emerge early. The spores released from the infected heads land on the later emerging florets and infect the developing seed. Infection during

flowering is favored byfrequent rain showers, high humidity and temperature. The disease is internally seed borne, where pathogen infects the embryo in the seed.



***Ustilago nuda* causing Loose smut of wheat disease showing disease cycle**

Control measures

Sow certified seed of wheat varieties that are resistant to loose smut and recommended for your area by your nearest Extension adviser. None of the wheat varieties are resistant to allthe physiologic races of the loose smut fungus, however some are moderately to highly resistant.

If you grow a variety susceptible to loose smut, be sure to plant certified seed purchased from a reliable dealer. Certified seed carries a minimum amount of infection. Only wheat fields that meet rigid specifications with respect to disease will pass certificationrequirements. Competent inspectors closely examine fields of all growers who apply for seed certification to make sure that no loose smut, or other serious seedborne wheat diseases are present.

The best insurance against loose smut is seed treatment with a fungicide containing carboxin or triadimenol systemic fungicides applied to the seed. These

fungicides have the unique ability of being taken up by the germinating seed. They check or kill the loose smut fungus within the seed while controlling surface-borne bunt or covered smut and a number of fungi that cause weedling blights (damping-off). Carboxin is sold under various trade names often in combination with another fungicide. These mixtures give excellent smut control and also provide protection against a wide range of fungi that attack the germinating seed and young seedling.

The hot-water soak technique for ridding wheat seed of the loose smut fungus, while highly effective, is difficult to use and often reduces the germination percentage and vigor of the wheat seed. This procedure should be attempted only by experienced personnel with the necessary equipment.

Conclusion

Fungi are a group of such plants which lack root system and chlorophyll. Hence, they lack thecapacity of mineral absorption and photosynthesis. They obtain their nutrition from other living or dead plants and animals. Most fungi are saprophytic, i.e., they obtain their nutrition from dead organic matter. There are certain fungi which obtain their nutrition from living cells. These are known as parasitic fungi. These fiungi also affect the physiological processes of the host. The toxins produced by certain fungi cause morphological deformity in the host. Such effects of the parasite appear in the host plant in the form of disease symptoms. The disease caused by these fungi include late blight of potato, Red rot of sugarcane, Loose smut of wheat etc., which can be controlled by number of methods, i.e. chemically or biologically.

References

Butler, E.J. 1973. Fungi and Disease in Plants, Intern, Book Distributers.Dehradun.

David S. Ingram , 1999. Plant Disease. Harper Collins Publishers, London United Kingdom.

Mehrotra, R.S. and Aneja, R.S. 1998. An Introduction to Mycology.New Age IntermediatePress.

Mishra, A.K. and Bohra, A. 2005. Plant Pathology: Disease and Management, Publ. AgrobiosJodhpur, pp714.

Sambamurty, A.V.S.S. 1992. A Text book of Plant Pathology.I.K. International Pvt. Ltd.504p.

Singh V, Pandey PC and Jain DK. 2005.A Text Book of Botany, Rastogi Publ. Meerut.

Singh, R.S. Principle of Plants Pathology. Oxford and IBH Publ. Co. New Delhi

Singh, R.S.983. Plants Diseases.Oxford and IBH Publ. Co. New Delhi.

Strobel, G.A. and D.E., Mathre 1970. Outlines of Plant Pathology. Van NostrandReinholdCo. New York.

Tarr, S.A.J. 1972. The Principle of Plants Pathology. Winchester Press, New York.

Western, J.H. 1971. Diseases of Crop Plants.McMillan Press London.