Fundamentals of Plant Pathology (HPI 100) 3 (2+1)

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1. Fundamentals of Plant Pathology (HPI 100) 3 (2+1)

Introduction to the science of phytopathology, its objectives, scope and historical background. Classification of plant diseases, symptoms, signs, and related terminology. Parasitic causes of plant diseases (fungi, bacteria, viruses, phytoplasma, protozoa, algae and flowering parasitic plants), their characteristics and classification. Non-parasitic causes of plant diseases. Infection process. Survival and dispersal of plant pathogens. Plant disease epidemiology, forecasting and disease assessment. Principles and methods of plant disease management. Integrated plant disease management.

Practical: *Familiarity with general plant pathological laboratory and field equipments. Study of disease symptoms and signs and host parasite relationship. Identification and isolation of plant pathogens. Koch's postulates. Preparation of fungicidal solutions, slurries, pastes and their applications.*

Lecture -1

INTRODUCTION TO THE SCIENCE OF PHYTOPATHOLOGY: ITS IMPORTANCE, SCOPE AND CAUSES OF PLANT DISEASES

Aim: To acquaint the students with the science of phytopathology; its objectives, general concepts and classification of plant diseases

Science of Phytopathology or Plant Pathology and Its Importance

- Plant protection has been accepted as broad area of research and technology at the national level by the Indian Council Agricultural Research, New Delhi; and Plant Pathology is an important discipline of Plant Protection.

Plant Pathology- Definition

- Plant Pathology, also known as Phytopathology is a branch of agricultural, biological or botanical science which deals with the study of diseases in plants - their causes, etiology, epidemiology, resulting losses and management.

Relation to other Sciences

- Plant pathology is related to many other sciences such as virology, mycology, bacteriology, microbiology, physiology, chemistry, genetics, biotechnology etc., all of which provide the knowledge required for the correct diagnosis and management of plant diseases.

Objectives of Plant Pathology

- To study living, non-living and environmental causes of diseases or disorders of the plants.
- To study the mechanism of plant disease development.
- To study interaction between host/susceptible and the pathogens.
- To develop systems of management of plant diseases and reducing losses caused by them.

Importance of Plant Diseases or Plant Pathology

- Losses they cause.

- About 34% of the crop produce is lost annually due to diseases, insect-pests and weeds on the global basis (Cramer, 1967); out of which, 12% is lost due to diseases (caused by fungi, bacteria or viruses), 11% due to nematodes, 7% due to insect-pests and 3% due to weeds.

- When plant protection measures are not implemented, annual loss of 30-50% are common in major crops including horticulture (Encyclopedia Britannica, 2002)

Epidemics

- Late blight of potato caused by *Phytophthora infestans* was responsible for causing **Irish famine** in 1845 by destroying the potato crop, the staple food of the people.
- Hundreds of thousand people died of hunger and disease, and there was a large scale migration of the population to other countries including North American continent.
- The population of Ireland was 8 million in 1940, which was reduced to 4 million after the famine.
- This single disease forced man to realize the importance of plant diseases, and brought the **science of Plant Pathology to lime light**.

Other Famines

- **Wheat rust** epidemics occurred from time to time in many countries. Wheat rusts forced farmers to change their cropping pattern and wheat was replaced by corn or maize or rye.
- Brown spot of rice caused by *Helminthosporium oryzae* was responsible for **Bengal famine in 1943**, which many people think one of the reasons for the division of Bengal
- **Coffee rust** caused by *Hemileia vastatrix* forced to cut down the coffee plants in Sri Lanka in 1867.
- **Powdery mildew** of grapevines caused by (*Uncinula necator*), by 1854, reduced the French wine production by 80 per cent.
- In 1878, the **downy mildew** caused by *Plasmopara viticola* ultimately led to the discovery of **Bordeaux mixture.**

Losses in India

- Wheat rusts cause a loss of Rs. 400 crore annually.
- In the years of epidemics, losses are Rs. 5000 crore or more.
- Loose smut of wheat is estimated to cause an average loss of 3 per cent (about Rs. 500 crore) every year.
- Other plant diseases such as red rot of sugarcane, potato viruses, rice blast and blight, Karnal bunt of wheat, root knot of tomato, eggplant and cucurbits, apple scab, mango malformation, bunchy top of banana and sandal spike are responsible for huge losses.

Effect on Society

- Infected grains or the fruits may contain toxins (such as aflatoxin, fumonosin) which cause insanity, paralysis, stomach disorder and liver cancer.
- The money spent on the management of plant diseases is also a loss because in the absence of diseases this money could be saved.
- There are many other implications on the transport and agro-based industry in the event of plant disease inflicted yield loss.
- There is restriction on the movements of food grains and other agricultural produce due to the threat of quarantine pathogens and pesticide residues in the produce causing further loss.

Causes of Plant Diseases

- Plant diseases are caused by a variety of pathogens.
- The word **pathogen** can be broadly defined as any agent or factor that incites 'pathos' or disease in an organism. Thus in strict sense, the pathogens do not necessarily belong to living or animate groups.

Abiotic (Inanimate) factors

- They include mainly the deficiency or excess of nutrients, light, moisture, aeration, abnormality in soil condition, atmospheric impunities etc. Examples are: Black tip of mango (due to SO_2 toxicity), khaira disease of rice (due to Zn deficiency), whiptail of cauliflower (Mo deficiency), hollow and black heart of potato (due to excessive accumulations of CO_2 in storage), bitter pit of apple (due to Ca deficiency).

Mesobiotic causes

- These are the disease incitants which are neither living nor non-living. They are considered to be on the threshold of life. They are:
- **Viruses**: They are infections agents made up of one type of nucleic acid (RNA or DNA) enclosed in a protein coat. Examples of viral diseases of plants are: potato leaf roll, leaf curl of tomato and chillies, and mosaic disease of many plants.
- **Viroids**: They are naked, infectious strands of nucleic acid. They cause diseases like potato spindle tuber, citrus exocortis, chrysanthemum stunt, cadang cadang of coconut palm, star crack of apple, etc.

Biotic (Animate) causes

This category includes the pathogens which are animate or living or cellular organisms. They are:

- **Prokaryotes** like bacteria which are unicellular prokaryotic microorganisms lacking true nucleus. Examples of diseases caused by true bacteria are: brown rot or wilt of potato, soft rot of potato and vegetables, , citrus canker, etc.

i) **Phytoplasma** are wall-less prokaryotes and cause diseases like peach X.
ii) **Fastidious bacterium**, *Xylella fastidiosa* causes almond leaf scorch, Pierce's disease of grapevine.

- **Eukaryotes** are the organisms with true nucleus.

i) **Fungi:** Potato wart, powdery mildew, rust, smuts, red rot of sugarcane (nearly 80% of plant diseases are caused by fungi).

ii) **Straminopiles** (Oomycetes): Downy mildews, late blight of potato, white rust of crucifers, damping off etc.

iii) **Protozoa**: Hart rot of coconut palm and phloem necrosis of coffee.

iv) **Algae**: Red rust of mango or papaya or litchi

v) **Metazoan animals** (Nematodes): Root knot of vegetables, ear cockle of wheat, citrus decline etc.

vi) **Parasitic flowering plants** (Phanerogamic plant parasites): *Dodder, Striga, Orobranche, Loranthus, Phoradendron,* etc.

Lecture -2

HISTORY OF PLANT PATHOLOGY (EARLY DEVELOPMENTS AND ROLE OF FUNGI IN PLANT DISEASES)

Aim: To acquaint the students with the history of plant pathology (early developments and role of fungi in plant diseases)

ANCIENT HISTORY

- Since organized agriculture developed 4000 years ago, special attention was given to plant diseases and pests. Symptoms such as blight, wilt, root rot, etc. were known to the people at that time.
- In Rig Veda, germ theory of disease was also advocated and the men of learning in Vedic period (Ca. 1500-500 BC) were aware that the diseases were caused by microorganisms. While this fact was not perceived by majority of the scientists in Europe only until 120 years ago.
- Vraksha Ayurveda, a book written by Surapal in 11th century in Ancient India is the first book in which much light has been thrown on plant diseases. The diseases have been divided in 2 groups: i). Internal (probably physiological disorders), and ii) External (probably infectious diseases).
- Symptoms of plant diseases are also mentioned in old testaments like Bible, Shakespear's poems and dramas and other Christian literature. Rust, smut, mildew and blights are very often quoted in Bible.
- In his book, "Enquiry into Plants", Theophrastus (286 BC), a Greek philosopher, recorded his experiences about plant diseases in a book. His experiences were based on imagination and not on experimentation.
- After Theophrastus, no definite opinion could be formed about plant diseases for the next 2000 years, although diseases continued to harm the crops and puzzle scientists of those days.
- During this period, the plant diseases were attributed to many causes which included divine power, religious belief, superstitions and effects of stars and wrath of God, etc.
- The Romans used to celebrate a festival called 'Robigalia' to ward off rust. It is reported to be initiated by the king Numa Pompilus around about 700 BC to please the 'Rust God' Robigo and continued with modifications in the Christian era.
- The association of barberry with stem rust of wheat has been recognized by the farmers for centuries. In 1660, the farmers in France secured passage of law which required destruction of barberry bushes in that area. Similar laws were made and executed strictly in United States in early 18th century.

- Antony van Leeuwenhoek observed the microorganismic cells for the first time under microscope in 1674.
- The Italian botanist Pier Antonio Micheli, also known as Father of Mycology first observed fungi and saw their spores. He also demonstrated that if spores of these fungi are placed on the pieces of fruit, they grew into new thalli of the fungus. Though this was a successful experiment, it was not accepted universally.
- He wrote a book entitled, '*Nova Plantarum Genera*' in 1729. Many of such early researches were not accepted because of the strong belief of the people in the 'Theory of Spontaneous Generation' in which microorganisms spontaneously or automatically originate from inanimate or non-living matter.
- In 1743, Needham, an English Clergyman and naturalist upheld strongly this theory. To prove his point, he boiled meat broth in flask which was corked.
- In 1775, Lazarro Spallanzani, an Italian investigator challenged Needham and set out to prove that decay of meat broth by bacteria and other lower forms of life could be prevented by heating the material in a flask which was sealed off in such a way as to exclude contamination from the air. He, however, did not receive recognition and was refuted by opponents who did not get the results due to their faulty techniques.
- Carl von Linne (Linnaeus), a Swedish scientist wrote a book entitled, "*Species Plantarum*" in 1753 and is credited with giving binomial nomenclature.
- C.H. Persoon's "*Synopsis Methodica Fungorum*" (1793) is the chief starting point for the nomenclature of the Uredinales, Ustilaginales and Gasteromycetes.
- E.M. Fries (1821-1832) was a Swedish scientist and is regarded as 'Linnaeus of Mycology'. His *Systema Mycologicum* is the chief starting point for the nomenclature of fungi.
- In 1775, Tillet, a French botanist published a paper on bunt or stinking smut of wheat. He proved that such wheat seeds that contained a black powder on their surface produced more diseased plants than the clean seeds. He believed that the disease was caused by some poisonous substance produced by the black powder. He also observed a reduction in disease incidence when the seeds were treated with salt and lime. Therefore, it can be said that he was an experimenter who lived ahead of his times.
- Felice Fontana, an Italian physicist and naturalist published a paper in 1767 in which he expressed the view that grain rust was a distinct parasitic entity. He distinguished the red and black stages of wheat rust and made microscopic drawings.
- Benedict Prevost, who was Swiss Professor of Philosophy at the Academy of France, in 1807 discovered the life cycle of bunt fungus. He studied germination of wheat bunt

fungus spores and conceived the idea that this organism penetrated the young wheat plant and was the actual cause of the disease.

- Prevost's experiments provided the first proof and interpretation of the role of microorganisms in the causation of the disease. He also demonstrated the control of smut by steeping seeds in a solution of copper sulphate.
- Tulasne brothers (R.L. and C. Tulasne) of France who have produced illustrated descriptions of rust and smut fungi, had also confirmed the findings of Prevost.
- In 1845, late blight devastated the potato crop in Ireland and other parts of Europe which attracted the attention of mycologists and plant pathologists to plant diseases. Much of early literature on this disease is found in 'Gardener's Chronicle'.
- M.J. Berkeley who was the most prominent British mycologist was at first somewhat conservative in supporting of the parasitic theory, but advocated it strongly in 1846.
- Montague in France in 1845 described the causal fungus as *Botrytis infestans*. However, the fungus was real cause of this disease and not the result was proven experimentally by Speers and Schneider in 1857 and Anton de Bary (1861, 63).

Modern Experimental Plant Pathology

- The foundation of modern experimental plant pathology was laid by the German scientist Heinrich Anton de Bary (1831-1888). He made a great contribution to the understanding of science of Plant Pathology and is suitably regarded as Father of Modern Plant Pathology. His major contributions are:
- He confirmed the findings of Prevost in 1853.
- In 1861, he experimentally proved that *Phytophthora infestans* was the cause of late blight of potato. He is credited with the ultimate proof of the organisms being plant pathogens.
- He studied other diseases like rusts, smuts, downy mildews and rots.
- He reported the heteroecious nature of rust fungus in 1885.
- He also reported the role of enzymes and toxins in tissue degradation caused by *Sclerotinia sclerotiorum* in 1886.
- de Bary was first to report that lichen consists of a fungus and an alga, and coined the term symbiosis.
- He studied in detail the life cycle of downy mildew fungi and their parasitism.
- His well known text book "*Morphologie und Physiologie der Pilze Flechten und Myxomyceten*" written in 1866 and 1884 records the broad classification of fungi.

- He trained a large number of students from all over the world who came to his laboratory.
- More than 60 of them became prominent in field and carried his techniques.
- Famous among them were M.S. Woronin of Russia, who studied club root of crucifers caused by *Plasmodiophora brassicae*; P.A. Millardet of France; HM ward of England, who studied coffee rust in Sri Lanka and gave 'bridging host theory', Fallow of U.S.A., who spent his long active career as cryptogamic botanist, and provided leadership in study of parasitic fungi; and A. Fisher of Switzerland worked on bacterial Plant Pathology and is known for the infamous Fisher –Smith controversy regarding the role of bacteria in causing plant diseases.
- J.G. Kuhn, who was a contemporary of de Bary and his countryman, was, initially a farm manager. He contributed significantly to the studies on infection and development of smut in wheat plant and development and application of control measures, particularly seed treatment for cereals.
- He wrote the first book on Plant Pathology "Diseases of Cultivated Crops, Their Causes and Their Control" in 1858 in which he recognized that plant diseases are caused not only by an unfavourable environment, but can also be caused by parasitic organisms such as insects, fungi and parasitic plants.
- The theory of spontaneous generation was a major impediment in the development of science of Plant Pathology, which was finally disproved by Louis Pasteur who established the 'germ theory of the disease' in case of anthrax in relation to man and animals. It changed the way of thinking of scientists and led to a tremendous progress.
- Significant impetus to this progress was added by Robert Petri, who developed artificial nutrient media for culturing of microorganisms and Brefeld (1875,1883,1912) who contributed greatly to Plant Pathology by introducing and developing modern techniques for growing microorganisms in pure culture.
- Robert Koch (1876) who was a Germen Physician and co-worker of Pasteur established that for proving that a certain microorganism was the cause of some infectious disease, certain necessary steps (Koch's postulates) must be carried out and certain conditions must be satisfied. They are:
- A specific organism must always be associated with the disease.
- The organism has to be isolated in pure culture.
- The organism must produce specific disease in a healthy susceptible host when the latter are inoculated with it.
- The organism must be re-isolated from the experimental (diseased) host in pure culture and its identity be established and it must have the characteristics as the organism in step 2.

Lecture-3

HISTORY OF PLANT PATHOLOGY (ROLE OF OTHER PLANT PATHOGENS)

Aim: To acquaint the students with history of Plant Pathology (Role of other Plant Pathogens)

Bacteria as Plant Pathogens

- In 1882, T.J. Burrill of USA for the first time reported that a plant disease (fire blight of apple and pear) was caused by a bacterium (now known as *Erwinia amylovora*).
- Wakker (1883) showed that yellows disease of hyacinth was also caused by a bacterium.
- E.E. Smith of USA is regarded the most outstanding and main contributor to the discovery of most of plant diseases due to bacteria since 1895. He is considered Father of Phytobacteriology for his discoveries and methodologies.
- Smith’s name is still remembered as he resolved the controversy with the German scientist A. Fischer (1897, 99) who did not agree that bacteria were the causes of diseases in plants.
- Smith was also among the first to notice and study the crown gall disease (1893, 1894). He considered crown gall similar to cancerous tumors of humans and animals.
- Later in 1977, it was demonstrated by Chilton and his team that the crown gall bacterium, *Agrobacterium tumefaciens* transforms the normal plant cells in tumour cells by introducing into them a part of plasmid which becomes inserted into the plant cell chromosome DNA.

Viruses

- Virus diseases of plants have a long history. Among many diseases of unknown cause, potato leaf roll, as ‘leaf curl’ gave concern in the 2nd half of 18th century and broken tulips were illustrated by painters 200 years before that.
- There were many methods of transmission of leaf mottling of jasmine and passion flower by grafting.
- In 1886, Adolf Mayer, a Germen Director of Agricultural Experiment Station at Wageningen, Netherland, introduced the term ‘mosaic’ and showed that the mosaic was infectious and the juice from infected plants if applied to the healthy plants could reproduce the disease.
- In 1891, Smith showed that the peach yellows was contagious disease and could be bud transmitted.
- In 1892, Dimitri Ivanowski proved that the causal agent of tobacco mosaic disease could pass through bacteria proof filters.

- In 1898, Beijerinck, (Father of Plant Virology) a distinguished Dutch microbiologist, demonstrated that the causal agent of tobacco mosaic could diffuse through an agar- agar membrane and concluded that the tobacco mosaic was caused by a non-corpuscular "contagium vivum fluidum" (or contagious living fluid) and called founder of virology and it's a virus.

- Stanley (1935) obtained a crystalline protein by treating the juice of the tobacco mosaic infected leaves with ammonium sulphate, which when placed on the healthy leaves could produce the disease symptoms. It was the first major contribution regarding the nature of the viruses and was awarded Noble Prize for it.

- In1936, Bawden and Pirie discovered the real nature of the *Tobacco mosaic virus* and demonstrated that the crystalline preparations of the virus actually consisted of not only proteins but also small amount of nucleic acid (RNA).

- In 1939, Kausche *et al.* viewed first *Tobacco mosaic virus* particles under electron microscope.

- Finally in 1956, Gierrer and Schramm showed that the nucleic acid fraction of the virus is actually required for infection and multiplication in the host and protein coat provided the protective covering to it.

Viroids

- In 1971, Diener and Raymer reported that the potato spindle tuber disease was caused by a small (250-400 base pair long), single stranded circular molecule of infectious RNA, which he called a viroid.

- Viroids seem to be the smallest nucleic acid molecules to infect plants but no viroid has so far been found in animals.

- Since then a dozen more viroids have been reported.

- In 1982, a circular single stranded viroid-like RNA (300-400 base pairs long) was found encapsidated together with the single stranded linear RNA (about 4500 base pairs long) of velvet tobacco mottle virus. This small circular RNA was called Virusoid which seems to form an obligatory association with the viral RNA in many plant viruses.

Phytoplasma and Rickettsia like Organisms

- Doi *et al.* (1967) and Ishiie *et al.* (1967) independently observed Mycoplasma Like Organisms (MLOs) now called as phytoplasma in the phloem of plants exhibiting yellows and witches' broom symptoms (earlier thought to be caused by viruses).

- The number of plant diseases of phytoplasma etiology is large. Some examples are aster yellows, mulberry dwarf, potato witches' broom and sandal spike.

- These organisms resembling mycoplasma could not be isolated and cultivated on artificial cell free media and they have shown more relatedness to acholeplasma than the mycoplasma, and are called phytoplasma.

- Later in 1973, some of the mycoplasmas such as the causal agent of citrus stubborn and corn stunt diseases could be grown in cell free media, and were helical in morphology and had motile stages and were named as spiroplasma. The agent *Spiroplasma citri* causing citrus stubborn is the type species of the genus and *S. kunkelli* causes corn stunt.

Fastidious Vascular Bacteria

- Some organisms were also observed in grapevines infected with Pierce's disease, in peach infected with phony peach and others.

- More recently such diseases have been reported to be caused by fastidious vascular xylem limited bacteria *Xylella fastidiosa,* and phloem limited bacteria *Candidatus librobacter.*

- Examples of xylem inhabiting fastidious bacteria causing diseases: Pierce's disease of grapevine, citrus variegated chlorosis, almond leaf scorch.

- Examples of phloem inhabiting fastidious bacteria are: Club leaf of clover, citrus greening, yellow vine disease of watermelon, bunchy top of papaya.

Flagellate Protozoa

- In 1909, Lafont observed flagellate protozoa in the latex bearing cells of laticiferous plants of Euphorbiaceae family without causing any harm to their hosts.

- However, in 1931, Stahel found the flagellates infecting phloem of coffee plants and causing abnormal phloem formation and wilting of trees.

- Vermeulen in 1963 presented additional and more convincing evidence of the pathogenicity of flagellates to coffee trees and in 1976, flagellates were also found in the phloem of coconut palm trees infected with the hart rot disease.

History of Plant Pathology in India

- The development of science of Plant Pathology in the modern era in India as in other countries followed the development of mycology. The study of fungi in India was initiated by Europeans in the 19^{th} century. They used to collect fungi and send the specimens for identification to the laboratories in Europe.

- During 1850-1875, D.D. Cunningham and A. Barclay started identification of fungi in India itself. Cunningham made a special study of rusts and smuts.

- K.R. Kirtikar was the first Indian scientist who collected and identified the fungi in the country.

- E.J. Bulter who is also known as the ‘Father of Plant Pathology’ in India, initiated an exhaustive study of fungi and diseases caused by them in 1901 at Imperial Agricultural Research Institute at Pusa (Bihar).
- During his stay of 20 years in this country, he made a scientific study of mostly fungal plant diseases known in India at that time. The diseases studied by him for the first time included wilt of cotton and pigeon pea, different diseases of rice, toddy palm, sugarcane, potato and rusts of cereals.
- He wrote a monograph on ‘Pythiaceous and Allied Fungi’; and a classic text book, ‘Fungi and Diseases in Plants’ in 1918.
- J.F. Dastur (1886-1971), a colleague of Butler, was the first Indian Plant Pathologist who is credited with a detailed studies of fungi and diseases in plants.
- He studied the genus *Phytophthora* and diseases caused by it in castor and potato. He is internationally known for the establishment of *Phytophthora parasitica* from castor.
- G.S. Kulkarni published exhaustive information on downy mildew and smuts of sugarcane and pearl millet.
- B.B. Mundkur started work on control of cotton wilt through varietal resistance.
- He was also responsible for the identification and classification of large number of Indian smut fungi.
- His most significant contribution to plant pathology will be remembered through the ‘Indian Phytopathological Society’ which he started almost single handedly in 1948 with its journal ‘Indian Phytopathology’.
- He also authored a text book entitled, ‘Fungi and Plant Diseases’.
- Dr. K.C. Mehta of Agra College, Agra investigated the life cycle of cereal rusts in India during the first half of 20th century.
- Dr. R. Prasada trained by Dr K.C. Mehta continued the work on rusts and added to the knowledge of linseed rust.
- Luthra and Sattar (1953) developed the solar heat treatment of wheat seed for the control of loose smut. SN Dasgupta carried out exhaustive studies on black tip of mango.
- T.S. Sadasivan worked out the mechanism of wilting in cotton due to *Fusarium oxysporum* f. sp. v*asinfectum.*
- M.K. Patel, V.P. Bhide and G. Rangaswami pioneered the work on bacterial plant pathogens in India.
- M.J. Thirumalachar conducted exhaustive studies on rusts and smuts, and developed a number of antibiotics for controlling plant diseases in India.

- Afterwards, Plant Pathology became a major subject in various agricultural colleges and universities and organized research was conducted on major plant diseases affecting crop plants in India.

- Notable contributions included the works of B.L. Chona on sugarcane diseases and Agnihothrudu in tea diseases, R.K. Agrawala on apple diseases and G.S. Saharan on oilseed plant diseases to name a few.

Lecture 4

GENERAL CONCEPTS AND CLASSIFICATION OF PLANT DISEASES

Aim: To acquaint the students with general concepts and classification of plant diseases

Definitions and Concepts

Disease: According to Horsfall and Diamond (1959), disease may be defined as a malfunctioning process that is caused by continuous irritation by a pathogen and/or environmental factor resulting in some suffering producing symptoms.

Disorder: The diseases caused by the deficiency of nutrients or unfavourable environmental are sometimes termed as disorders or physiological disorders.

Pathogen: It is the agent responsible for inciting 'pathos' i.e. ailment or damage.

Parasite: These are the organisms which derive the food materials needed for their growth from other living organism (the host). All the pathogens are parasites but all the parasites are not pathogens. As some of the parasites live on their hosts without causing any damage to them as symbiotic relationships, e.g., *Rhizobium* bacterium in legume roots, mycorrhizae and lichens.

Biotrophs are the organisms which regardless of the ease with which they can be cultivated on artificial media obtain their food from living tissues only in nature in which they complete their life cycle). They were earlier also called **obligate parasites,** e.g., rusts, smuts, powdery mildews etc.

Saprophytes/saprobes are the organisms which derive their nutrition from the dead organic matter. Some parasites and saprophytes may have the faculty or (ability) to change their mode of nutrition.

Facultative saprophytes are ordinarily parasites which can grow and reproduce on dead organic matter under certain circumstances. They are also called **hemibiotrophs** which attack the living tissues in such a way as biotrophs but continue to grow and reproduce after the tissues is dead.

A parasite is called **necrotroph** when it kills the host tissue in advance of penetration and then lives saprophytically, e.g. *Sclerotium rolfsii* and *Pythium* species. Similar to necrotrophs are **facultative parasites** which live as saprophytes but under favourable conditions they can attack living plants and become parasites. The necrotrophs are also known as **perthotrophs** or **perthophytes**.

Pathogenicity is the ability of a pathogen to cause disease under a given set of environmental conditions. Whereas, **pathogenesis** is the chain of events that leads to development of a disease in the host.

Parasitism is a phenomenon by which a plant parasite becomes intimately associated with the plant; it draws nutrition and multiplies and grows at the expense of the plant host.

Virulence is a measure or degree of pathogenicity of an isolate or race of the pathogen. The term **aggressiveness** is often used to describe the capacity of a pathogen to invade and grow in the host plant and to reproduce on or in it. This term like virulence is used as measure of pathogenicity.

Immunity of a plant against a disease is absolute quality. It denotes the freedom of plant from disease, when the pathogen cannot establish parasitic relationship with the host. High resistance and low susceptibility approach immunity.

Disease resistance is the ability of an organism to overcome completely or in some degree the effect of a pathogen or other damaging factor; whereas susceptibility in the inability of the plant to resist the effect of the pathogen or other damaging factor.

Hypersensitivity is the extreme degree of susceptibility in which there is rapid death of the cells in the vicinity of the invading pathogen. It halts the further progress of the pathogen. Thus, hypersensitivity is a sign of very high resistance approaching immunity.

Infection is the establishment of the parasitic relationship between the pathogen and host following entry or penetration.

Incubation period is the time elapsing between penetration and completion of infection i.e. development of the disease symptoms.

Invasion and colonization is the growth and multiplication of the pathogen through the tissue of the host varying extent.

Effects of Disease

- The diseased plants do not function or look normal showing structural abnormality and / or physiological disorder and can not grow, develop and reproduce to its genetic potential.

Classification of Plant Diseases

Based on plant part affected

- **Localized,** if they affect only specific organs or parts of the plants.
- **Systemic,** if entire plant is affected. or

They can be classified as root diseases, stem diseases, foliage/foliar diseases, etc.

Based on perpetuation and spread

- **Soil borne** -when the pathogen perpetuates through the agency of soil.
- **Seed borne** -when the pathogen perpetuates through seed (or any propagation material).
- **Air borne** -when they are disseminated by wind e.g. rusts and powdery mildews.

Based on the signs and symptoms produced by the pathogens

- Diseases are classified as rusts, smuts, powdery mildews, downy mildews, root rots, wilts, blights, cankers, fruit rots, leaf spots, etc. In all these examples, the disease are named after the most conspicuous symptom of the disease appearing on the host surface.

Based on the host plants affected

They can be classified as cereal crop diseases, forage crop diseases, flax diseases, millet diseases, plantation crop diseases, fruit crop diseases, vegetable crop diseases, flowering plant diseases, etc.

Based on major Causes

They can be classified as fungal diseases, bacterial diseases, viral diseases, mycoplasmal diseases, etc.

Based on Infection Process

- **Infectious** -All the diseases caused by animate causes, viruses and viroids can be transmitted from infected host plants to the healthy plants and are called infectious.

- **Non-infectious**- Non-infectious diseases can not be transmitted to a healthy plant. Also referred as non-parasitic disorders or simply physiological disorders, and are incited by abiotic or inanimate causes like nutrient deficiency or excess or unfavorable weather conditions of soil and air or injurious mechanical influences.

Classification of Animate Diseases in Relation to Their Occurrence

- **Endemic diseases** -which are more or less constantly present from year to year in a moderate to severe form in a particular geographical region, i.e. country, district or location.

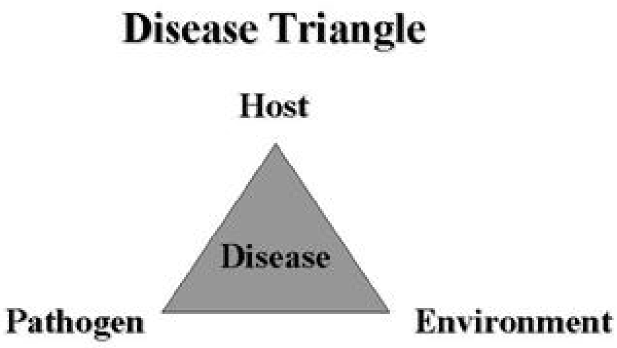
- **Epidemic or epiphytotic diseases** -which occur widely but periodically particularly in a severe form. They might be occurring in the locality every year but assume severe form only on occasions due to the favourable environmental conditions occurring in some years.

- **Sporadic diseases** occur at irregular intervals and locations and in relatively few instances.

- **Pandemic diseases**: A disease may be endemic in one region and epidemic in another. When epiphytotics become prevalent through out a country, continent or the world, the disease may be termed as pandemic.

Disease triangle

- The interaction of the host, the pathogen and the environment results in disease development. It is generally illustrated by a triangle, also called a disease triangle.



Disease development is dependent on three factors: host, pathogen and environment. The area within the triangle is the interaction of these components and represent the amount of disease.

Disease Development in Plant Population

This is determined by:

- **Host**: All conditions in host that favour susceptibility.
- **Pathogen**: Total of virulence, abundance etc.
- **Environment**: Total of conditions that favour the pathogen and predispose the host plants to pathogen attack.
- **Time:** Specific point of time at which a particular event in disease development occurs and the duration or length of time during which the event takes place.

'*Effective disease control or measures aim at breaking this E-H-P triangle*'.

Lecture -5

SYMPTOMS AND SIGNS OF PLANT DISEASES

Aim: To acquaint the students with symptoms and signs of plant diseases

Symptoms and signs

Symptoms -External expression or the evidence of the abnormalities in the appearance of the diseased plants brought about by the pathogens after host-pathogen interaction.

Sign- When the pathogen itself becomes visible on the host surface in the form of its organs or structures. eg. sclerotia, mycelium etc.

Disease syndrome- A sum total of variety of symptoms produced by the disease.

I. Symptoms of Plant Diseases Due to the Character and Appearance of Visible Pathogen, its Structures and Organs

i. Mildews

- Mildews consist of white, grey, brownish or purplish pathogen growth on the host surface.
- **Downy mildew** is characterized by a tangled cottony or downy growth mostly on the lower surface of the leaves or other plant parts.
- **Powdery mildew** consists enormous number of spores are formed on superficial growth of the fungus giving a dusty or powdery appearance on the host surface. Black minute fruiting bodies may also develop in the powdery mass.

Grapevine downy mildew **Pea powdery mildew**

ii. Rust

- Rust appears as relatively small pustules of the spores, usually breaking through the host epidermis.

- Pustule is a small blister-like elevation of the epidermis, often opening to expose spores. The pustules may be dusty or compact, and red, brown, yellow or black in colour.

Pea rust

iii. Smut

- Smut means a sooty or charcoal like powder.
- The affected parts of the plants show black or purplish black dusty areas.
- Symptoms usually appear on floral organs, particularly the ovulary areas.
- The pustules on the leaves and stems are usually larger than those of rusts.

iv. White Blister

- White blister-like pustules appear on the leaves and other parts of cruciferous plants

which break open the epidermis and expose powdery masses of spores.

- Such symptoms are called ‘white rust’, although there is nothing common with them and the rusts.

White blisters on a crucifer

v. **Blotch**

- It consists of superficial growth giving the affected plant parts i.e., fruits and leaves smoky (blotched) appearance, e.g. sooty blotch of apple.

Sooty blotch of apple

vi. **Sclerotia**

- A sclerotium is a compact, often hard mass of dormant fungus mycelium.
- Sclerotia are mostly dark in colour and are found mixed with the healthy grains as in the case of ergot of wheat and rye.

vii. **Exudation**

- Mass of bacterial cells ooze out on the surface of the affected organs where they may be seen as a drop or smear in several bacterial diseases such as bacterial blight of paddy, gummosis of stone fruits and fire blight of apple and pear.
- They form crusts after drying.

viii. **Mycelial growth**

- Appearance of white cottony, mycelial growth of the fungi like *Dematophora necatrix* on affected roots of apple is an important diagnostic feature of white root rot in the field.

Sclerotium rolfsii **sclerotia**

White root rot of apple

Mango gummosis

II. Symptoms Resulting from Internal Disorders in the Host Plants

i. Colour change

- **Discolouration** is **c**hange of colour from normal. It is one of the most common symptoms of plant diseases. The green pigment of leaves disappears entirely and is replaced by yellow pigments.
- **Etiolation** is yellowing due to the lack of light.
- **Chlorosis** is yellowing due to low temperature, lack of iron, excess of the lime or alkali in soil and infection by viruses, fungi and bacteria.
- **Albilinism** is the phenomenon in which the leaves become devoid of any pigment and look bleached or white.

Mosaic symptoms on a cucurbit leaf

- **Chromosis** is change of colour to red, purple or orange.

ii. **Overgrowths or hypertrophy**

- **Hypertrophy** is the abnormal increase in the size of the plant organs due to increase in the size of the cells of a particular tissue, whereas
- **Hyperplasia** is the abnormal increase in the size of the plant organs due to increase in the number of cells of which the tissue or organ is composed, owing to increased cell division.

The overgrowths cause galls, curl, pockets or bladders, hairy root, witches' broom, intumescence etc.

Crown gall of peach

iii. **Atrophy or Hypoplasia or Dwarfing**

- Atrophy is inhibition of growth and thereby showing stunting and dwarfing effectmon the plants.
- The whole plant may be dwarfed or only certain organs are affected. e.g. rice dwarf, phony peach etc.

III. Necrosis

- Death of the cells, tissues and organs occurs as a result of parasitic activity.
- The characteristic appearance of the dead areas differs with different hosts, host organs and with different parasites.
- Necrotic symptoms include spots, streaks or stripes, canker, blight, damping off, burn, scald or scorch and rot.

Colocasia blight

Brown rot of pear

IV. Wilt

- Characterized by drying of the entire plant.
- Leaves and other green or succulent parts loose their turgidity, become flaccid and droop down.
- Usually seen first in some of the leaves.
- Later, the young growing tip or the whole plant may dry up.
- May be caused by injury to the host system or the conducting vessels.
- Wilting due to disease is different from the physiological wilting where the plant recovers as soon as the supply of water is retained.

Fusarium wilt of pea

Bacterial wilt of capsicum

V. Die-back or Wither Tip

- Symptoms are characterized by drying of plant organs, especially stems or branches, from the tip backwards.
- It is also a form of necrosis caused directly by the pathogen or its toxins.

Die-back symptom on mango

Lecture-6

GENERAL CHARACTERISTICS OF FUNGI AND FUNGAL-LIKE ORGANISMS CAUSING PLANT DISEASES

Aim: To acquaint the students with general characteristics of fungi and fungal-like organisms causing plant diseases

Fungi

- Fungi are eukaryotic, spore bearing achlorophyllous organisms with absorptive nutrition that generally reproduce both sexually and asexually and whose somatic structures known as hyphae are surrounded by cell wall containing chitin and glucans (but no cellulose) as the skeletal components.

Oomycetes

- A group of fungal like organisms, the O*omycota* generally referred to as (Oomycetes), until about 1990 were called considered as lower fungi.
- Majority have cell wall composed of glucans and small amount of cellulose, but not chitin.
- Now regarded as members of the kingdom *Chromista* (also known as *Straminopila*) rather than *Fungi*; but continued to be discussed with fungi because of their many other similarities to them, especially the way they cause disease in plants**.**

Habitat

- Most of the more than 1,00,000 known fungus species are strictly saprophytic and they live on dead organic matter.
- About 50 species cause diseases in humans
- About 50 species cause diseases in animals.
- More than 10,000 species of fungi can cause diseases in plants.
- All plants are affected by some kinds of fungi and each of the parasitic fungus can attack one or many kinds of plants.
- Some fungi like those causing rusts, smuts, powdery mildews and downy mildews can grow and multiply on their host plants during their entire life, and therefore called as **obligate parasites or biotrophs**.
- Fungi like *Venturia inaequalis*, the apple scab fungus, pass a part of their life on the host as parasites and a part on the dead tissues of the same host on the ground as saprophytes in order to complete their life cycle in nature, and therefore are called **hemibiotrophs**.

- **Facultative saprophytes** generally grow parasitically on the hosts but continue to live, grow and multiply on the dead tissues of the host and may further move out of the host debris into the soil or other decaying plant material to grow and multiply strictly as saprophytes, e.g., *Phytophthora infestans.*

- **Facultative parasites** live perfectly well in the soil or elsewhere as saprophytes but can parasitize or cause disease in the plants when they get opportunity under favourable conditions to do so, e.g., *Alternaria alternata*.

Morphology

- **Mycelium**: A filamentous vegetative body.

- **Hypha (pl. hyphae):** Individual branch of mycelium which are generally uniform in thickness, usually about 2-10 µm in diameter. The hyphae may be septate or aseptate

- **Coenocytic hyphae** - The aseptate or non-septate hyphae having the nuclei scattered in the cytoplasm.

- **Septate hyphae-** The hyphae have septa having perforations through which cytoplasmic strands, containing nuclei can migrate from one cell to the other.

- A characteristic **dolipore septum** is formed in certain basidiomycetes.

- Diameter of hyphae may be as narrow as 0.5 µm and as wide as 100 µm.

- The vegetative thallus may consist of only **one cell** or may even be **naked**, amoeboid, multinucleate **plasmodial mass without cell wall** or a system of strands of varying diameter called **rhizomycelium**.

- In some fungi, hyphae form aggregates and develop various kinds of structures. These may be:

- **Rhizomorphs**: thicker root like aggregates.

- **Sclerotium**: a hard roundish or amorphous structure and has a hard rind surrounding a soft interior i.e. medulla.

- **Stroma**: some fungi also develop mat like structures which contain the fruiting bodies.

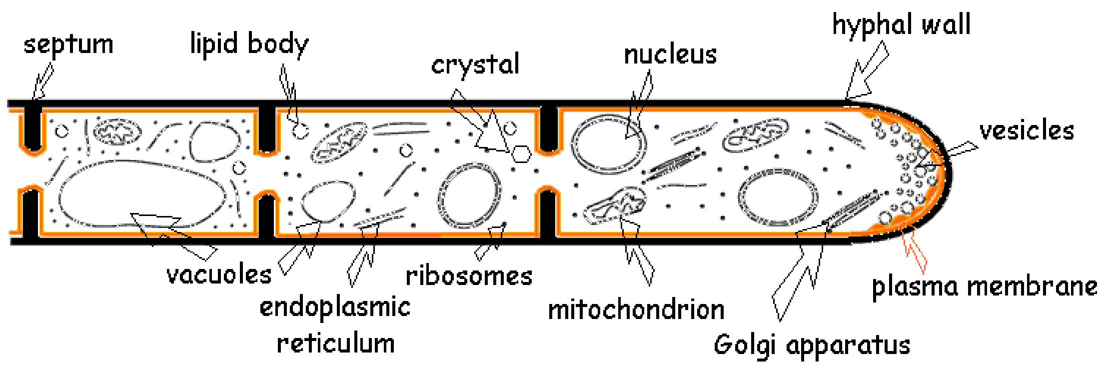
- Rhizomorphs and sclerotia help the fungus to survive from one cropping season to the other and also function in initiating the disease as a primary inoculum.

- **Pseudoparenchyma**: Sometimes the hypahe aggregate to form tissue like structure called **plectenchyma**. In cross section, it appears like parenchymatous cells of the higher plants. This is called pseudoparenchyma and consists of rounded fungal cells.

- **Prosenchyma**: Less compact structures consisting of hyphae made of elongated cells. These are found mostly in the stroma or fruiting bodies of Ascomycota or Basidiomycota.

Fungal Cell Structure

A typical fungal cell consists of protoplasm which is surrounded by a cell membrane, and cell wall being its outer most covering. The protoplasm typically contains nucleus, mitochondria, ribosomes, golgi bodies and endoplasmic reticulum among others.



Typical fungus hyphal structure

Cell wall

- Made up of chitin and β-glucans in the members belonging to the kingdom **Fungi**.
- Chitin is a polymer of N-acetyl glucosamine units which is also found in the exoskeleton of insects.
- Made up of cellulose in kingdom **Straminopila** (including Oomycota).
- Cellulose is a polymer of β D-glucose units and is also found in the cell wall of plants and algae.

Nucleus

- The nuclei of fungi are extremely small and lie near the limit of resolution power of light microscope.
- Electron microscopic studies have revealed that the nuclear membrane does not disappear but constricts in the centre like a dumb-bell during nuclear division. This type of division is known k**aryochoresis,** term given by Moore in 1964.
- In **meiosis**, however, the nuclear membrane disappears.

Life Cycle

- The fungi are mostly **haploid** organisms, i.e. their nuclei are haploid.
- The **thallus** multiplies by asexual methods.

- After sexual reproduction, a **diploid zygote** is formed.
- The zygote represents the diploid phase which is terminated by meiosis forming **haploid spores**.
- The spores on germination form the **haploid somatic** phase.
- In *Ascomycota* and *Basidiomycota*, **plasmogamy** is not immediately followed by **karyogamy**.
- The two nuclei of opposite strains remain as paired nuclei called **dikaryon** and give rise to **dikaryotic hyphae**.
- These are of limited duration in *Ascomycota*, as only a small portion of the mycelium, viz., the **ascogenous hyphae** become dikaryotic.
- In *Basidiomycota*, these extend through the major portion of the life cycle. The **monokaryotic mycelium** is of short duration and its function is only to establish dikaryotic mycelium as soon as possible.
- The dikaryotic phase, which is of **short duration** in *Ascomycota* is **extensive** in the life cycles of *Basidiomycota*.

Basic Types of Life Cycles Found in Fungi (depending on the extent of haploid, diploid or dikaryotic phases of the mycelium)

- **Asexual cycle**: Found in all anamorphic (=imperfect fungi)
- **Haploid cycle**: *Mucor* , *Rhizopus*, etc.
- **Haploid cycle with restricted dikaryophase**: *Eurotium*, *Talaromyces*
- **Haploid dikaryotic cycle**: *Ustilago maydis*
- **Dikaryotic cycle**: *Ustilago tritici*, *Puccinia graminis*
- **Haploid- diploid cycl**e: *Saccharomyces cerevisiae*
- **Diploid cyc**le: *Pythium*, *Phytophthora*, *Albugo*

Lecture 7

REPRODUCTION IN FUNGI AND FUNGAL LIKE ORGANISMS CAUSING PLANT DISEASES

Aim: To acquaint the students with reproduction in fungi and fungal like organisms causing plant diseases

Reproduction

Fungi may reproduce in three ways:

- Vegetative reproduction
- Asexual reproduction
- Sexual reproduction

I. Vegetative Reproduction

It occurs through:

- Fragmentation of hyphae
- Production of thin walled spores such as oidia or arthrospores
- Production of swollen, thick walled spores with storage of rich food, i.e. chlamydospores which are formed to tide over the adverse environment.
- **In Fragmentation,** a bit of broken hyphae establishes a new colony. Fragmentation occurs in nature and is usually employed in the laboratory to keep the fungus growing by transferring small portions of hyphae to new culture tubes.
- **In Budding,** small soft portion of the cell wall bulges out and a daughter nucleus migrates into it. Then the bud is pinched out by a constriction at the point of its origin. Sometimes the budding is so quick that a chain of buds is formed due to non-detachment of the daughter buds, and is called **pseudomycelium**.
- **In Fission**, a cell divides in a transverse plane into two cells. Although, it is a characteristic of bacteria and it also occurs in fission yeasts.

II. Asexual Reproduction

- It occurs through internally or externally produced spores which also act as agents of dissemination, survival and infection.
- In *Straminopila* (*Oomycota)* and some *Fungi* (Zygomycota), asexual spores are produced endogenously inside a sac like structure called s**porangium** and are released either by rupture of sporangial wall or through a pore or opening in its wall.

- They are either motile with one or two flagella called **zoospores** or non-motile **aplanospores**.
- Sporangia are formed on specialized hyphal branches called **sporangiophores**.
- **Conidia** are another type of asexual spores which are cut off terminally or laterally from specialized hyphal branches called **conidiophores**.
- Conidiophores may be unbranched or may branch variously, both monopodially or sympodially and conidia are produced singly or serially in chains on these branches.
- While conidiophores of most fungi remain free, in some they appear to be aggregated and often compactly arranged to form a variety of characteristic structures such as **coremia, synnema**, **sporodochia, acervuli** and **pycnidia**. These are primarily designed to provide large number of spores within a small space available to the fungus.
- The locomotory appendages or flagella of zoospores are of two types, i.e. whiplash and tinsel.
- The whiplash flagella are much thinner at the tip.
- The tinsel type flagella, which are found only in the members of kingdom *Straminopila (Oomycota)* have large number of small hair like outgrowths called **mastigonemes** or **flimmers** on their entire length.

Structure of Flagella

- The flagellum of fungi has a typical 9+2 structure as in case of other eukaryotes. In 9+2 structure, the flagellum is composed of 9 peripheral pairs of fibrils surrounding the two central fibrils (hence the name 9+2). The two central fibrils are attached to the **blepharoplast** lying inside the motile cell. The membrane surrounding the 9+2 fibrils is continuous with the plasma membrane of the cell. Sometimes, a threadlike **rhizoplast** connects the blepharoplast to the nucleus**.**
- The bacterial flagella lack this 9+2 structure and are made of 8 rows of **flagellin** (protein) molecules twisted around each other like a rope.

III. Sexual Reproduction

The sexual reproduction in fungi and other similar micro-organisms involves:

- **Plasmogamy**-. fusion between two sexual cells.
- **Karyogamy-** fusion of the nuclei. It results in the formation of a diploid nucleus, which immediately or later undergoes **meiosis** to form 4 haploid nuclei.

Fungi achieve plasmogamy by a variety of methods:

i. G**ametogamy**

ii. G**ametangiogamy,**

iii. **Spermatization**

iv. **Somatogamy.**

i) Gametogamy: It is the fusion (or copulation) between gametes.

- Gametes are naked wall- less sex cells which copulate to form a zygote.
- If two gametes are similar in size, they are called **isogametes** and their copulation is called **isogamy**.
- Copulation between two dissimilar gametes, one smaller (male) and the other bigger (female) is called **anisogamy**.
- The fusion between motile male gamete and non- motile female gamete (oosphere or egg) lying in the oogonium is called **heterogamy**.

ii) Gametangiogamy: It is the fusion between gametangia (or the sex organs) when gametangia are similar in shape and size, these are called **isogametangia** and are designated as (+) and (-) gametangia rather than male and female.

- When the gametangia are different in shape and size, they are called **heterogametangia**.
- The male is usually smaller and club shaped while the female is bigger and globular.
- Fusion between two similar gametangia results in a zygote which is called a **zygospore**.
- The zygote formed by the fusion between morphologically distinct gametangia is called **oospore** and the process **oogamy**.
- The plasmogamy between them is called **gametangial copulation** or **contact**.

- **Gametangial copulation** is of two types:
  - ✓ The entire gametangia fuse, the intervening wall disappears and their contents come to lie in the common cell formed by their fusion, e.g., *Mucor*.
  - ✓ The contents of the male gametangia migrate into the female gametangium through a pore or fertilization tube and the male gametangium is left empty, e.g., *Rhizophidium*.
- **Gametangial contact**: The male nuclei and not the cytoplasm of antheridium migrate into the oogonium through a pore dissolved at the point of contact or through a fertilization tube formed by the antheridium. e.g. *Pythium*, *Phytophthora*, *Albugo*.

iii. Spermatization: It occurs in *Ascomycota* and *Basidiomycota*.

- Spermatia (sing. Spermatium), minute male gametes, are formed like conidia on spermatiophores.

- The spermatiophores may be formed exogenously or inside a spermogonium e.g. *Puccnia.*
- The spermatium when comes in contact with the female gametangium (or the receptive hyphae) releases the male nucleus into the female gametangium through a pore.

iv. Somatogamy:

In this, sex organs are not formed and somatic cells as such act as gametangia and fuse together. e.g. *Agaricus.* Somatogamy may occur between cells of the same hypha (in a homothallic fungus) or between cells of the different thalli (in a heterothallic fungus). **Anastomosis**, which is the fusion of hyphae is frequent in *Ascomycota* and *Basidiomycota*.

IV. Parasexual Reproduction

- The anamorphic (=imperfect) fungi lack sexual reproduction involving karyogamy and meiosis. But the genetic recombination in this case is achieved through the parasexual method.
- In this, the haploidization takes place by aberrant mitosis, whose frequency is, however, very low. It was first discovered by Pontecorvo and Roper in 1952 in *Aspergillus nidulans*.

Lecture -8

CLASSIFICATION OF FUNGAL PLANT PATHOGENS

Aim: To acquaint the students with classification of fungal plant pathogens

- **Traditionally,** fungi were classified under Thallophyta division of Plantae Kingdom under 4 classes, viz., phycomycetes (also called lower fungi including *Oomycetes, Chytridiomycetes and Zygomycetes*), ascomycetes, basidiomycetes and deuteromycetes (including *fungi imperfecti*).

- **Ainsworth's classification** (1966, 1973) included kingdom *Fungi* with Myxomycota and Eumycota divisions. The Eumycota division was divided into 5 sub-divisions, viz., mastigomycotina, zygomycotina, ascomycotina, basidiomycotina and deuteromycotina which were further sub-divided into (classes, orders and so on).

- Classification system based on information on phylogenetic relationships of fungi obtained by small submit (18 S) ribosomal RNA gene comparison are now used. **The classification given by Hawksworth *et al*. (1995) and partially modified by Kirk *et al.* (2001) is now universally accepted.** According to it, the fungi are placed in three kingdoms: *Fungi*, *Protozoa* and *Straminopila*

- All the taxa (kingdom to species) are now italicized in print and underlined when hand written.

There are standard endings for the various taxa. Phylum ends in -mycota, class in –mycetes, sub-class in -mycetidae, order in –ales, family in –aceae, and genus and species have no fixed endings.

- Genus is always written with the first letter in capital (proper noun).

- Species in small letters as adjective qualifying the noun.

Kingdom *Fungi*

They are characterized by:

- Unicellular or filamentous somatic phase
- Cell wall made of chitin and glucans
- Absorptive mode of nutrition
- Presence of only whiplash type of flagella
- Mitochondria with flattened cristae, and

(6) Presence of peroxisomes and golgi bodies

There are four Phyla in Kingdom *Fungi: Chytridiomycota*, *Zygomycota*, *Ascomycota* and *Basidiomycota*

- **The imperfect fungi that reproduce only asexually and were earlier placed in Deuteromycotina (Ainsworth, 1973) are no more accepted as a distinct taxonomic category**. They are not monophyletic unit, but are fungi which are anamorphs (asexual stages) of *Ascomycota* and *Basidiomycota*.

- Those which have not been shifted to *Asco-* or *Basidiomycota* are placed under **mitosporic** or **anamorphic** fungi.

Kingdom *Straminopila*

These are characterized by:

- Unicellular or filamentous somatic phase
- Cell wall made of cellulose
- Absorptive mode of nutrition
- Presence of tinsel type of flagella
- Tubular mitochondrial christae
- Presence of peroxisomes and golgi bodies

They showed closer genetic relationship to brown algae and diatoms rather than the *Fungi* in 18 S r RNA phylogenetic studies.

There are 3 phyla: *Hyphochytridiomycota, Labrynthulomycota*, *Oomycota*.

Kingdom *Protozoa*

These are characterized by:

- The somatic phase is plasmodial or amoeboid.

- There is no cell wall in the somatic phase.

- Nutrition is by ingestion except in plant parasites (e.g., *Plasmodiophoromycota*), where the plasmodium obtains nutrition by absorption.

- Mitochondrial cristae are tubular.

There are 4 phyla: *Acrasiomycota, Dictyosteliomycota, Myxomycota, Plasmodiophoromycota.*

OUTLINE CLASSIFICATION OF FUNGI

Pseudofungi

Kingdom 1: ***Protozoa*** (uni-cellular microorganisms)

Phylum i: *Myxomycota*

Class: *Myxomycetes* (plasmodial slime moulds, do not infect plants)

Order: *Physarales*

Genera: *Mucilago*, *Fulago*, *Physarum* causing slime moulds in low lying plants)

Phylum ii: *Plasmodiophoromycota* (endoparasitic slime moulds)

Class : *Plasmodiophoromycetes*

Order: *Plasmodiophorales*

Genera: *Plasmodiophora*, *P. brassicae* causing club root of crucifers, *Polymyxa*, *P. graminis* on wheat and other cereals, and can transmit viruses, *Spongospora*, *S. subterranea* causing powdery scab of potato tubers

Kingdom 2: ***Chromista/ Straminopila*** (microorganisms unicellular or multi-cellular)

Phylum i: *Oomycota* (zoospores flagellate)

Class: *Oomycetes* (mycelium non-septate, sexual spores- oospores, asexual spores- zoospores)

Order: *Peronosporales*

Family: *Peronosporaceae*, (the downy mildews, sporangia borne on sporangiophores of determinate growth, obligate parasites)

Genera: *Plasmopara*, *P. viticola* causing downy mildew of grapes, *Peronospora*, *P. tabacina* causing downy mildew/ blue mould of tobacco), *Bremia*, *B. lactucae* causing downy mildew of lettuce, *Pseudoperonospora*, *P. cubensis* causing downy mildew of cucurbits; *Sclerospora*, *S. graminicola* causing downy mildew/ green ear disease of pearl millet

Family: *Pythiaceae* (sporangia, usually zoosporangia are produced along somatic hyphae, or tips of the hyphae of indeterminate growth and set free. OSogonia thin walled. Facultative parasites)

Genera: *Pythium*, *P. debaryanum* causing damping off of seedlings; *Phytophthora*, *P. infestans* causing late blight of potato

Family: *Albuginaceae* (the white rusts, sporangia borne in chains)

Genus: *Albugo, A. candida* causing white rust of crucifers

True Fungi

Kingdom 3: ***Fungi*** (produce mycelium, the walls of which contain glucans and chitin)

Phylum i: *Chytridiomycota* (produce zoospores with single posterior flagellum)

Class: *Chytridiomycetes*

Order: *Chytridiales*

Family: *Olpidiaceae*

Genera: *Olpidium*, *O. brassicae* being parasitic on roots of cabbage and other plants, and can transmit plant viruses; *Physoderma*, *P. maydis* causing brown spot of corn; *Synchytrium endobioticum* causing potato wart

Phylum ii: *Zygomycota* (produce non-motile spores in sporangia. Sexual spores are zygospores)

Class: *Zygomycetes* (bread moulds)

Order: *Mucorales*

Family: *Mucoraceae*

Genera: *Rhizopus*, *R. stolonifer* causing soft rot of fruits and vegetables; *Choanephora*, *C. cucurbitarum* causing soft rot of squash; *Mucor* causing bread mould and storage rots of fruits and vegetables)

Order: *Glomales* (the endomycorrhizae)

Genera; *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora*

Phylum iii: *Ascomycota* (the sac fungi, produce ascospores)

Class i: *Archiascomycetes*

Order: *Taphrinales* (asci naked arising from binucleate ascogenous cells)

Family: *Taphrinaceae*

Genus: *Taphrina*, *T. deformans* causing peach leaf curl

Family: *Protomycetaceae*

Genus: *Protomces*, *P. macrosporus* causing stem gall of coriander

Class ii: *Saccharomycetes* (the yeasts, mostly unicellular fungi that reproduce by budding, asci naked, no ascocarps produced)

Order: *Saccharomycetales*

Family: *Saccharomycetaceae*

Genera: *Galactomyces* causing citrus sour rot; *Saccharomyces*, *S. cerevisiae*, the bread yeast

Class iii: *Plectomycetes* (ascomycetes with cleistothecia)

Order: *Eurotiales*

Family: *Eurotiaceae*

Genera: *Eurotium*, teleomorph of *Aspergillus*, causing bread mould and seed decay; *Talaromyces*, teleomorph of *Penicillium*, *P. expansum* causing blue mould rot of fruits

Order: *Erysiphales* (the powdery mildew fungi, asci formed in cleistithecia. mycelium, conidia, and cleistothecia produced on the host surface, obligate parasites)

Family: *Erysiphaceae*

Genera: *Blumeria*, *B. graminis* causing powdery mildew of cereals and grasses; *Erysiphe cichoracearum* causing powdery mildew of cucurbits; *Leveillula*, *L. taurica* causing powdery mildew of tomato and capsicum; *Podosphaera*, *P. leuchotricha* causing powdery mildew of apple; *Sphaerotheca*, *S. pannosa* causing powdery mildew of rose and peach; *Uncinula*, *U. necator* causing powdery mildew of grapes

Class iv: *Pyrenomycetes* (ascomycetes with perithecia)

Order: *Hypocreales*

Genera: *Hypocrea rubra* is the teleomorph of *Trichoderma viride*; *Nectria galligena* causing European canker of apple; *Gibberella fujikuroi* causing bakane/ foolish disease of rice; *Claviceps purpurea* causing ergot of grain crops

Order: *Microascales*

Genera: *Ceratocystis*, *C. fagacearum* causing oak wilt; *C. fimbriata* causing root rot of sweet potato, *C. paradoxa* causing butt rot of pineapple

Order: *Phyllachorales*.

Genera: *Glomerella*, *G. cingulata* (teleomorph of *Colletotrichum* sp.) causing many anthracnose diseases and bitter rot of apple; *Phyllachora graminis* causing leaf spot of grasses

Order: *Ophiostomatales*

Genera: *Ophiostoma*, *O. novo-ulmi* causing Dutch elm disease

Order: *Diaporthales*

Genera: *Diaporthe*, *D. citri* causing citrus melanose, *D. vexans* causing fruit rot of eggplant, *Gnomonia leptostyla* causing leaf blotch of walnut, *Cryphonectria* *parasitica* causing chestnut blight; *Leucostoma* (formerly *Valsa*) causing canker disease of peach and other trees

Order: *Xylariales*

Genera: *Hypoxylon*, *H. mummatum* causing severe canker of poplars; *Rosellinia*, *R. necatrix* causing root rot diseases of trees and vines; *Xylaria*, causing tree cankers and wood decay; *Eutypa*, *E. armeniacae* causing apricot canker

Class iv: *Loculoascomycetes* (ascomycetes with ascostroma or pseudothecium)

Order: *Dothidiales*

Genera: *Mycosphaerella*, *M. musicola* causing Sigatoka of banana, *M. fragariae* causing leaf spot strawberry; *Elsinoe*, *E. fawcetti* causing citrus scab, *E. ampelina* causing grape anthracnose

Order: *Capnodiales*

Genera: *Capnodium*, being one of the many fungi causing sooty moulds on the plants

Order: *Pleosporales*

Genera: *Cochliobolus*, the teleomorph of *Helminthosporium or Bipolaris* causing leaf spot diseases on many plants including *C. miyabeanus* causing brown spot disease of rice leading to the infamous Bengal famine, *Pleospora*, teleomorph of *Stemphylium*) causing black mould of tomato, *Leptosphaeria*, the teleo*morph* of *Phoma* causing black leg and foot rot of cabbage, *Venturia*, *V. inaequalis* causing apple scab and *V. pirina* causing pear scab, *Guignardia bidwelli* causing black rot of grapes

Class v: *Discomycetes* (ascomycetes with apothecia)

Order: *Rhytismatales*

Genera: *Lophodermium*, *L. seditiosum* causing pine needle cast, *Rhytisma acerinum* causing tar spot of maple leaves

Order: *Helotiales*

Genera: Monilinia, *M. laxa*, *M. fructigena*, *M. fructicola* causing brown rot of pome and stone fruits, *Sclerotinia*, *S. sclerotiorum* causing white mould and watery soft rot of vegetables, *Stromatinia*, *S. gladioli* causing corm rot of gladiolus, *Diplocarpon*, (the teleomorph of *Marssonina*), *D.*

maculatum causing black spot of quince and pear, *D. rosae* causing black spot of rose, *D. mali* leaf blotch and premature leaf fall of apple

Class vi: *Deuteromycetes* or mitosporic fungi (imperfect fungi): Sexual reproduction or structures rare, lacking or unknown.

Phylum iv. *Basidiomycota* (basidiomycetes, the club and mushroom fungi)

Class i: *Urediniomycetes* (rust fungi)

Order: *Uredinales*

Family: *Pucciniaceae*

Genera: *Puccinia*, *P. graminis tritici* causing black rust of wheat, *P. recondita* causing brown rust of wheat, *P. striiformis* causing yellow rust of wheat, *P. dianthii* causing rust of carnation. *Gymnosporangium*, *G. juniperi-virginianae* causing cedar apple rust; *Cronartium*, *C. ribicola* causing rust of pine; *Hemileia*, *H. vastatrix* causing coffee rust; *Melampsora*, *M. lini* causing flax rust; *Phragmidium*, *P. mucronatum* causing rose rust; *Uromyces*, *U. appendiculatus* causing rust of bean

Class: *Ustilaginomycetes* (smuts and bunts)

Order: *Ustilaginales*

Family: *Ustilaginaceae*

Genera: *Ustilago*, *U. tritici* causing loose smut of wheat, *U. maydis* causing corn smut, *U. nuda*, causing smut of barley; *Urocystis*, *U. cepulae* causing smut of onion

Family: *Tilletiaceae*

Genera: *Tilletia*, *T. caries and T. foetida* causing hill bunt of wheat, *T. (Neovossia) indica* causing Karnal bunt of wheat

Class: *Basidiomycetes* (earlier grouped under hymenomycetes and gasteromycetes) produce basidiospores, basidia and basidiocarps)

Order: *Exobasidiales* (no fruiting bodies or basidiocarps)

Family: *Exobasidiaceae*

Genera, *Exobasidium*, *E. vexans* causing blister blight of tea

Order: *Ceratobasidiales*

Genera: *Athelia*, the teleomorph of *Sclerotium*, *S. rolfsii* causing southern blight and root rot of many plants, *S. cepivorum* causing the white rot onion; *Thanatephorus, T. cucumrris* , the teleomorph of *Rhizoctonia solani,* causing root rot and web blight of many plants; Typhula, causing Typhula blight or snow mould of turf grasses

Order: *Agaricales* (the mushrooms)

Family: *Agaricaceae*

Genera: *Agaricus, A. bisporus* , the white button mushroom; *Armillariella (Armillaria) mellea* causing root rot of trees; *Marasmius,* causing fairy ring disease of turf grass, *Pleurotus,* causing white rot on logs, tree stumps and living trees; *Pholiota*, causing brown wood rot in deciduous forest trees

Family: *Pluteaceae*

Genus: *Amanita, A. muscaria,* the fly agaric is a poisonous mushroom

Family: *Lycoperdaceae*

Genus: *Lycoperdon*, the puff balls

Order: *Phallales*

Family: *Geastraceae*

Genus: *Geastrum,* the earth stars

Order: *Aphyllophorales*

Genera: *Chondrostereum, C. purpureum* causing the silver leaf disease of trees, *Corticium, C. salmonicolor* causing pink disease in many fruit and other trees; *Heterobasidion, H. annosus* causing root and butt rot of many trees; *Ganoderma, G. lucidum* causing root and basal rot in many trees; *Phelinus,* causing tree root rot and cubical rots in buildings; *Peniophora, P. gigantea* causing decay in coniferous logs and pulpwood; *Polyporus, P. squamosus* causing heart rot of many forest trees.

Lecture 9

GENERAL CHARACTERISTICS AND REPRODUCTION OF BACTERIAL PLANT PATHOGENS

Aim: To Acquaint the students with general characteristics and reproduction of bacterial plant pathogens

General Characteristics

Bacteria are second most important organisms which cause plant diseases.

- They are prokaryotic single celled mostly achlorophyllous organisms whose body is surrounded by cell wall and nuclear material is not surrounded by membrane.
- They lack membrane bound organelles such as mitochondria or plastids and also a visible endoplasmic reticulum.
- Most of the bacterial species are saprophytes living on dead organic matter. There are about 200 bacterial species which are plant pathogenic.
- Morphologically the bacteria are rod shaped (bacilli), spherical (cocci), spiral (spirilli), coma shaped (vibrios) or thread like (filamentous).
- *Streptomyces* has a filamentous branched hypha-like structure, sometimes mistakenly called as **ray fungi**; and mycoplasma have no definite shape due to lack of cell wall.
- In young cultures the rod shaped bacteria range from 0.6 to 3.5 µm in length and from 0.5 to 1µm in diameter (0.6-3.5 x 0.5-1 µm size).
- Single bacterium mostly appears as hyaline or yellowish white under the compound microscope, when grown on a medium, soon a colony is formed.
- The colonies of most of bacteria have a whitish or greyish appearance but some of them develop yellow, red or other colours.

Bacterial Cell Structure

- A bacterium has a thin, relatively tough, rigid **cell wall**, and a distinct three layered but thin cytoplasmic membrane.
- Most bacteria have a **slime layer** made up of viscous gummy material. Slime layer has bacterial immunological property.
- When the layer is thick and firm, it is called **capsule**.
- Generally plant pathogenic bacteria lack capsule but some of them like *Pseudomonas* and *Xanthomonas* produce slime.

- Slime layer is mostly composed of polysaccharides but may rarely contain amino sugars, sugar acids, etc.

Flagella

- Most of the plant pathogenic bacteria have delicate thread like **flagella**, which are usually longer than the cell
- They are the organs of locomotion.
- The arrangement of flagella on bacterial cell is an important taxonomic character that aid in bacterial classification.
- This arrangement may be
  - **Monotrichous**- with one polar flagellum
  - **Lophotrichous** -tuft of flagella at one end
  - **Amphitrichous**- at both the ends
  - **Peritrichous** - distributed all around the cell or surface.
  - **Atrichous**- bacteria lacking flagella.

Gram Staining

Bacterial species are often distinguished from one another by **Gram staining.**

- In this process, a bacterial smear is heat fixed on glass slide, stained with crystal violet and mordanted with iodine and finally rinsed with ethanol.
- When the bacteria retain the crystal violet stain after rinsing, the bacteria are called **gram positive**; and those which do not retain the stain are called **gram negative**.
- The later are then counter stained with pink colour safranin.
- The ability of bacteria to retain crystal violet stain or not, depends upon fundamental structure of cell wall.

Gram positive vs Gram negative bacteria

Gram Positive bacteria	Gram Negative bacteria
1. Cell wall is thicker and homogemous.	Cell wall is thinner and usually thin layered.
2. Contains lower content of lipids (5-10%)	Contains higher content of lipids (up to 40%)
3. Peptidoglycan comprises up to 90% of the cell wall and hence maximum lipid.	Peptidoglycan comprises only 10%.
4. Techoic acid present.	Techoic acid absent.
5. Cell wall has higher amino sugar content (10-20%)	Low content of amino sugars
6. Cell wall is simple in shape and is single layered.	Varying cell wall shape and is tripartite (3-layered).
7. Mesosomes more prominent.	Mesosomes less prominent.
8. Retains violet dye	Retains red dye
9. Examples: *Bacillus*, *Clavibacter*, *Streptomyces*	Examples: *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Agrobacterium*, *Xylella*

Bacterial Cell Structure

- The bacterial cell is surrounded by a cell wall composed of **peptidoglycan** consisting of chain of alternating N-acetyl muramic acid and N-acetyl glucosamine units cross linked by tetrapeptide and pentaglycine units.
- The cell wall allows the inward passage of nutrients and the outward passage of waste matter and digestive enzymes.
- All the material inside the cell wall constitutes the **protoplast**.
- The protoplast consists of a cytoplasmic or protoplast membrane, which determines the degree of selective permeability of various substances into and out of the cell.
- The cytoplasmic membrane of bacteria resembles those of eukaryotes, but also contains respiratory and other enzymes located in the bacteria.
- The **cytoplasm**, which is a complex mixture of proteins, lipids, carbohydrates, many other organic compounds, minerals and water.
- The nuclear material consists of large circular **chromosome,** composed of DNA.
- The chromosomal DNA makes up the main body of genetic material of the bacterium and appears as a spherical, ellipsoidal, dumb-bell or Y-shaped body in the cytoplasm, but without any membrane.
- Such nuclear material does not show meiosis and mitosis.

- Some species also have additionally single or multiple copies of smaller circular genetic material called **plasmids**.

- Plasmids can move from one bacterium to another and even from the bacterium to plants as in crown gall disease. This special property is being utilized with much success in genetic engineering for transformation of some desired genes from one plant to another by using it as vector.

Flagella

- In bacteria, flagella are the organs of locomotion.

- They are very delicate and fragile and cultures are to be handled carefully for their staining.

- The flagella vary from 10-12 nm in width which is smaller than wavelength of light, therefore, cannot be seen by ordinary staining.

- Mordants like potassium sulphate and mercuric chloride are generally precipitated on flagella making the width more for making them visible under light microscope.

Parts of a Flagellum

- **Filament**: It is the outermost region of flagellum, and is helical, composed of flagellin with a molecular weight of 30000-40000 and is synthesized in the cell, which moves to the hollow core of the flagellum to the tip. Flagellin is a protein with 14 amino acids and is characterised by higher content of aromatic amino acids and absence of cysteine in many cases.

- **Hook**: Filament is attached to hook which is wider than the flagellum. This is 45 nm wide and made up of different types of protein. The hook of gram positive bacterium is longer than that of gram negative bacteria.

- **Basal body:** The third part called basal body consists of small central rod which is inserted into a system of rings. The gram positive and gram negative bacteria are different in the number of rings. The inner pair of rings (S and M) are embedded in cell membrane and are formed in both gram positive and gram negative bacteria. L and P rings are formed only in gram negative bacteria. S and M rings are important for movement of flagella.

Pili

- In some bacteria, small hair like structures are also present which are called pili.

- These are shorter than the flagella and are thicker (3-15 nm in diameter).

- The term fimbriae is sometimes also used for pili, but the term pili is reserved for those which are involved in conjugation.

- They are made up of protein sub-units pilin of molecular weight of 70000.
- It consists of a helically coiled fibre with a central hole of 2 nm in diameter.
- Fimbriae may be involved in attachment, whenever there is infection. Both flagella and pili originate from cell membrane and extend outward through the cell wall.

Reproduction

Bacteria multiply at a phenomenal rate by the process of **fission or binary fission**.

- As the cytoplasm and cell wall undergo division into two, the nuclear material is organized into a circular chromosome like structure which ultimately duplicates itself and gets distributed equally into 2 newly formed cells.
- Similarly, plasmids also duplicate and come into 2 daughter cells.
- The duplication occurs rapidly, once every 20 minutes.
- As a result a bacterium like *Eschersia coli*, starting from one bacterium may produce 1 million bacteria in 10 hours.
- However, this number is not reached because of gradual limitations of nutrients and toxic metabolites. Still what is achieved normally is phenomenonal.
- Such prolificacy in multiplication must be of great advantage both in survival of bacterial pathogen, and also for successive plant infections.

Recombination

- The genetical recombination in bacteria has been noticed by the following sexual-like processes:
    - **Conjugation:** Conjugation occurs when two compatible bacteria come into contact and part of the chromosomal or non-chromosomal genetic material of one is transferred to the other and incorporated into the genome of later through conjugal zygote formation and breakage and reunion. It was first observed by Lederberg and Tatum (1956) in *E. coli*.
    - **Transformation:** It occurs when the bacterium is genetically transformed by absorption of genetic material of another compatible bacterium, secreated by or released in a culture during the rupture, and its incorporation into the genome of the former. It was first observed by Griffith (1928) in *Enterococcus pneumoniae*.
    - **Transduction:** When genetic material from one bacterium is carried by its phage (virus) to another bacterium that it visits next and the later is genetically transformed. It was first discovered by Zinder and Lederberg (1952) in *Salmonella***.**

Mycoplasma/PPLO's

Mycoplasma, earlier known as 'Pleuro Pneumonia Like Organisms' (PPLO's) were discovered to be associated with the disease **bovine pleuro pneumonia** and were described in one of the orders mycoplasmatales under Eubacteria.

- Mycoplasma represent a group of organisms that lack cell wall and contain a very small genome.
- Phylogenetically, they are closely related to clostridia, the gram positive bacteria.
- As per the requirement for their growth, they can be divided into those which require sterol (mycoplasma and spiroplasma); and those which do not require sterols (acholeplasma and thermoplasma).
- The mycoplasma cells are small, pleomorphic (of different shapes) and divide by budding. The colonies of mycoplasma on agar exhibit a characteristic 'fried egg' appearance because of the formation of dense central core surrounded by lighter circular spreading area.
- The growth of mycoplasma is not inhibited by penicillin or other antibiotics that inhibit cell wall synthesis. But they are sensitive to tetracycline.
- The *Spiroplasma* genus is important plant pathogenically and has cork screw shaped cells. They are motile and exhibit undulating or rotating movement. *Spiroplasma citri* has been associated with citrus plants, where it causes citrus stubborn disease and corn plants which causes corn stunt.

Phytoplasma

They were earlier called MLO's and were found to be associated with several yellows and witches' broom diseases after their discovery by Doi *et al.* in 1967.

- They are different from mycoplasma in the sense that they can not be cultured on synthetic media.
- The change in terminology from MLO's to phytoplasma occurred since the studies of DNA homology in the highly conserved genes encoding ribosomal RNA and ribosomal protein.
- It showed that the phytoplasma comprise a coherent group distinct from other prokaryotes. Their closest relatives are in the genus *Acholeplasma*.
- As they have not been cultured on artificial medium *in vitro* and characterized apart from their host, they are referred to *Candidatus* status.

- They are associated with about 200 plant diseases including aster yellows, apple proliferation, peanut witches' broom, peach-x-disease, rice yellow dwarf and elm yellows.
- They are phloem inhabiting organisms and are graft transmissible in nature, and can also be transmitted by leaf hoppers.

Phytoplasmas in plant cell

Bacteria as Plant Pathogens

- Bacteria are known to grow in a wide range of habitat.
- All the plant pathogenic bacteria are mesophilic (they can grow at a temperature of 20-35ºC); and remain in the host plants as plant parasites and only partly in plant residues or as saprophytes in soil.
- They enter the plants either through natural openings such as stomata, lenticels or hydathodes or through wounds.
- The presence of free water is essential for bacterial infection. Once inside the plant tissues, they multiply only if there is water or at very high humidity.
- They multiply in the intercellular spaces and produce pectolytic and other cell wall degrading enzymes, thereby creating more space to move inside the host tissue.
- They kill the host cells by the action of extracellularly released enzymes and toxins and subsequently invade the dead cells. Most of the bacterial pathogens are necrotrophs.
- Some are apparently biotrophs.
- Some species colonize the xylem vessels and because of their physical presence or the slime ultimately cause the plugging of the water conducting tissues and cause wilt symptoms.

- Plant pathogenic bacteria produce various types of symptoms in plants as are caused by fungal pathogens. They cause soft rot of vegetables and fruits, wilts, cankers, scabs and also over-growths.

Lecture-10

CLASSIFICATION OF BACTERIAL PLANT PATHOGENS

Aim: To acquaint the students with classification of bacterial plant pathogens

Traditionally bacteria have been included in ***Plantae*** kingdom under Thallophyta; however, Haeckel in 1966 proposed the kingdom ***Protista*** to include all unicellular organisms and placed various organisms of Thallophyta plants and Protozoa animals in ***Protista***. Later, the nucleus character was given more importance. Chatton proposed the most appropriate conceptual basis for taxa at the highest level by recognizing two general patterns of cellular organelles as prokaryotes and eukaryotes in 1937. Stanier (1969) considered prokaryotes as lower protists including blue green algae, myxobacteria and eubacteria; and eukaryotes as higher protists including algae, fungi and protozoa. Prokaryotae was recognised a separate kingdom. However, the correct concept is that of 5 kingdoms according to Whittaker (1969) including *Plantae*, *Animalia*, *Fungi*, *Protista* and *Monera* (Prokaryotes).

- In '***Bergey's Manual of Determinative Bacteriology'*** the phytopathogenic bacteria have been classified into three divisions:

- **Division I – *Gracilicutes***

  They include prokaryotes with thin cell walls consisting of outer membrane with fatty acid glycerol ester-type lipids and are usually gram negative. They do not form endospores.

- **Division II – *Firmicutes***

  It included prokaryotes with thick (firm) cell wall consisting of peptidoglycan and unit membrane but without any outer membrane. Some of them produce endospore. They are gram positive.

- **Division III – *Tenericutes***

  They lack cell wall and cells are enclosed by a unit membrane only. They include mollicutes or mycoplasma like organisms (now called phytoplasma).

Detailed Classification of Phytopathogenic Bacteria

Kingdom: *Prokaryotae*
Division I: *Gracilicutes*
Class: *Proteobacteria* (mostly single-celled, non-photosynthetic)

Family 1: *Enterobacteriaceae* (They are peritrichous bacteria)
Genus: *Erwinia*
E. amylovora causing fire blight of apple and pear
E. carotovora pv. *carotovora* causing soft rot of vegetables
E. carotovora pv. *atroseptica* causing black leg of potato

Family 2: Pseudomonadaceae
Genus: *Pseudomonas*
P. syringae pv. *syringae* causing stone fruit bacterial canker
P. syringae pv. *tabaci* causing wild fire disease of tobacco
Genus: *Ralstonia*
R. solanacearum causing bacterial wilt of solanaceous crops
Genus: *Xanthomonas*
X. campestris pv. *campestris* causing black rot of cabbage
X. campestris pv. *phaseoli* causing common bean blight
X. campestris pv. *vesicatoria* causing tomato bacterial spot
X. oryzae pv. *oryzae* causing bacterial leaf blight of rice
X. axonopodis pv. *citri* causing citrus canker

Family 3: Rhizobiaceae
Genus: *Agrobacterium*
A. tumefaciens causing crown gall of stone fruits
A. rhizogenes causing hairy root of apple
Family : Still unnamed
Genus: *Xylella*
X. fastidiosa [earlier called RLO's rickettssia like organisms] xylem-inhabiting causing Pierce's disease of grapevines, phony peach, almond leaf scortch
Candidatus liberobacter asiaticus, phloem-inhabiting causing citrus greening
Unnamed, latex-inhabiting, causing bunchy top disease of papaya

Division 2: Firmicutes
Class 1: Firmibacteria (Simple gram positive bacteria)
Bacillus subtilis – biocontrol agent
Class 2: Thallobacteria (Gram positive, branching bacteria)
Streptomyces scabies causing common scab of potato
Clavibacter michiganense pv. *sepedonicum* causing ring rot of potato

Clavibacter michiganense pv. *michiganense* causing bacterial canker of tomato

Curtobacterium (*Corynebacterium*) *flaccumfaciens* causing bacterial wilt of bean

Division 3: Tenericutes

Class: Mollicutes (wall less prokaryotes)

Family: Spiroplasmataceae

Spiroplasma citri causing citrus stubborn

Spiroplasma kunkelii causing corn stunt

Several organisms called phytoplasma have been reported to cause various yellows and witches' broom type diseases are included in this group and have been given *Candidatus* status for the time being due to the inability of their culturing.

Lecture 11

GENERAL CHARACTERISTICS AND CLASSIFICATION OF VIRAL PLANT PATHOGENS

Aim: To acquaint the students with general characteristics and classification of viral plant pathogens

Introduction

- Viruses are sub-microscopic, intracellular, infectious entities and are composed of nucleic acid and proteins.
- Some viruses attack humans, animals or both and cause diseases like mumps, measles, chicken-pox, polio, rabies etc; some others attack plants.
- In plants, tulip breaking was reported in 17th century.
- Adolf Mayer in 1886 first proved that the sap from tobacco leaves infected with mosaic could transmit the disease to healthy leaves.

General characteristics

Characteristics of viruses which separate them from other causes of plant pathogens are:

- They are acellular.
- They are sub-microscopic and intracellular.
- They lack lipid membrane system and energy production.
- They use host machinery for their replication.

Sturcture of virus

- **Virion** is a technical term used for the virus particle. A virion consists of nucleic acid surrounded by a protein coat.
- The nucleic acid is called ‘nucleoid’ which may be either de-oxyribonucleic acid (DNA) or ribonucleic acid RNA (mostly RNA in plant viruses), but never both; and forms the genome.
- The protein coat is called ‘capsid’. It consists of many subunits which are similar and occasionally dissimilar, and these subunits are called **capsomeres**.
- The combined genome and the capsid are called ‘nucleocapsid’.
- Some viruses possess an envelop around the protein coat which is made of virus proteins and host cell lipids. These viruses are called ‘**enveloped viruses’**.

- In many groups of viruses, there is an additional protein layer between the capsid and the nucleoid. This is called '**virus core'**.
- In addition to the typical nucleoprotein composition, some viruses have carbohydrates/ lipids / enzymes.

Nucleoid

- The nucleoid (nucleic acid component) is located internally within a protein coat.
- Only one type of nucleic acid, i.e. either RNA or DNA is found in a virus.
- The amount of nucleic acid in a virion varies from 1 to 50 per cent.
- Higher percentage of nucleic acid is associated with larger DNA viruses like bacteriophages; while low content is found in animal viruses.
- The nucleic acid is infectious part and contains the genetic information for the synthesis of proteins and its own replication; and their assembly into the virion.
- Most of the plant viruses contain RNA, with exceptions like *Cauliflower mosaic virus*.

Capsid

The capsid is a protein coat surrounding the nucleoid and has the following functions:

- It protects nucleic acid from unfavourable extracellular environment.
- It facilitates nucleic acid entry into the host cells.
- It is antigenic.
- As compared to nucleoid, the protein coat shows a complex structure and provides shape to the virus particles.
- It interacts with the vector for specific transmission.

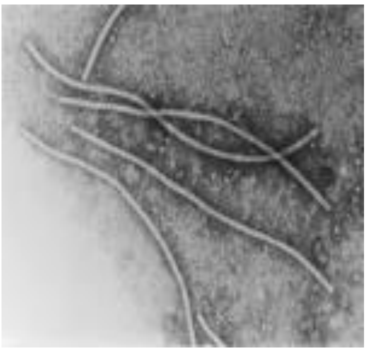
Morphology of Viruses

Viruses are of different shapes and sizes. They may be:

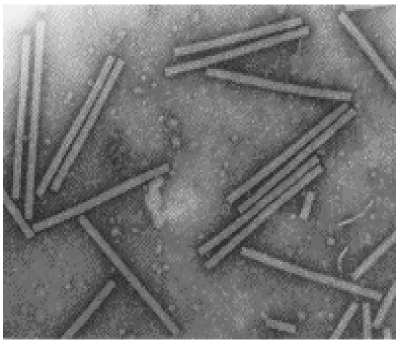
i. Elongated (rigid rods or flexuous threads)

ii. Spherical (isometric or polyhedral)

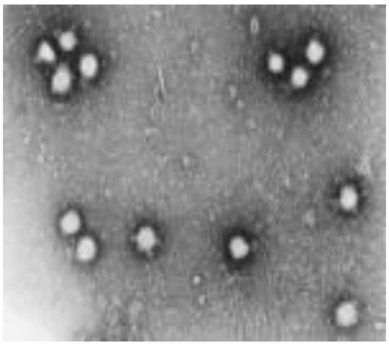
iii. Cylindrical (bacillus-like rods)



Flexuous rod-shaped virus



Rigid rod-shaped virus



Polyhedral virus particles

- Some elongated viruses are rigid rods about 15 x 300 nm in size, but most appear as long, thin, flexible threads that are usually 1-10 nm wide and 480-2000 nm in length.
- Rhabdoviruses are short bacillus-like cylindrical rods approximately three to five times as long as they are wide (52-75 x 300-380 nm in size).
- Most spherical viruses are actually polyhedral, ranging in diameter from about 17 nm (*Tobacco necrosis satellite virus*) to 60 nm (*Wound tumor virus*).
- *Tomato spotted wilt virus* is surrounded by a membrane and has a flexible, spherical shape about 100 nm in diameter.
- Many plant viruses have spilt genome consisting of two or more distinct nucleic acid strands encapsidated in different-sized particles made of the same protein subunits. For example: **Bipartite**, *Tobacco rattle virus* consisting of two rods, a long one (195 x 25 nm) and a shorter one (43 x 25 nm) and **Multi-partite** *Alfalfa mosaic virus*, consisting of four components of different sizes.

Composition and structure of viral proteins

- Viral proteins, like all proteins, consist of amino acids.
- The sequence of amino acid within a protein, which is encoded by the sequence of nucleotides in the genetic material, determines the nature and properties of the protein.
- For example, the protein subunit of *tobacco mosaic virus* consists of 158 amino acids in a constant sequence and has a mass of 17,600 daltons.

Composition and structure of viral nucleic acids

- Nucleic acid of most plant viruses consists of RNA, but now, a larger number of viruses have also been shown to contain DNA as its genome.
- Both RNA and DNA are long chain-like molecules consisting of hundreds or most of them thousands of units called nucleotides.
- Each nucleotide consists of a ring compound called the base attached to a five-carbon sugar (ribose in RNA, de-oxyribose in DNA), which in turn is attached to phosphoric acid.

- The sugar of one nucleotide reacts with the phosphate of another nucleotide, which is repeated many times, thus forming the RNA or DNA strand.
- In viral RNA, only one of four bases, adenine, guanine, cytosine and uracil can be attached to each ribose molecule. The first two, adenine and guanine, are purines and interact with the pyrimidines, uracil and cytosine.

Virus Infection and synthesis

- Plant viruses enter cells only through the wounds made mechanically or made by vectors, or are deposited into an ovule by an infected pollen grains.
- In a RNA replication of an RNA virus, the nucleic acid (RNA) is first freed from the protein coat.
- It induces the host cell to form the viral RNA polymerase.
- The RNA polymerase utilizes the viral RNA as a template and forms complementary RNA.
- The first new RNAs produced are not the viral RNAs but are the mirror images (complementary copies) of that RNA.
- As the complementary RNA is formed, it is temporarily connected to the viral strand. Thus, the two form a double-stranded RNA that soon separates to produce the original virus RNA and the mirror image (-) strand, with the latter then serving as a template for more virus (+) strand RNA synthesis.
- In dsDNA viruses, the viral ds DNA enters the cell nucleus and appears to become twisted and supercoiled and forms a minichromosome.
- The latter is transcribed into two single-stranded RNAs.
- The smaller RNA is transported to the cytoplasm, where it is translated into virus-coded proteins
- The larger RNA is also transported to the same location in the cytoplasm, but it becomes encapsidated by coat protein subunits and is used as a template for the reverse transcription into a complete virion dsDNA.

Spread of viruses

- For successful infection, viruses move into the adjacent cell through plasmodesmata intracellularly with cytoplasmic streaming.
- Some viruses move through host parenchyma cells, causing mostly local lesions.

- Many viruses move over fairly long distances through phloem specifically through sieve tubes and spread systemically and often quite rapidly in their host plant through vascular streams.
- Such movement is mostly through phloem but rarely xylem transport also occurs, e.g., *Lettuce necrosis yellows virus* and *Southern bean mosaic virus*.
- Once the virus enters the phloem, it moves rapidly into the sieve tubes towards the growing regions and other food utilizing parts of the plant.
- Virus movement is mostly upwards. Once the virus reaches the phloem, it spreads systemically throughout the plant and then moves to adjacent parenchyma cells through the plasmodesmata. This systemic distribution of viruses affects all parts of host plant except the apical meristem in some cases.

Transmission of Viruses

- Viruses must be brought in contact with the contents of living host cells. They achieve this quite effectively by transmission from an infected plant to a healthy plant in a number of ways.

Through vegetative propagation

- The viruses are transmitted from the infected plant parts to the healthy ones of the same generation and it results in only primary infection and occurs in monocyclic diseases.
- Examples are mosaics and leaf roll of potato, and sugarcane viruses.

Mechanical transmission through sap

- Some viruses can transmit from diseased to healthy plants through the mechanical transmission of the infected sap by touch.
- Virus infected sap adhering to tools, implements, insect mouth parts, and body, hands, clothes of man etc. can transmit viruses to the wounded plant cells.
- This type of mechanical transmission is wide spread in *Tobacco mosaic virus* (TMV), *Potato virus X*, etc.

Seed transmission: Majority of seed transmitted viruses are carried internally.

- Virus may be carried to the seed from the infected ovule or the pollen.
- A small percentage show seed transmission.
- Examples are *muskmelon mosaic virus* in watermelon, *barley stripe mosaic virus*, *tobacco ring spot virus* in soybean, *common bean mosaic virus*.

Pollen transmission

- Pollen transmission of virus occurs in sour cherry infected with *Prunus necrotic ring spot virus.*

Insect transmission

- Aphids, leaf hoppers, white flies, mealy bugs and scale insects constitute the insect vectors. However, aphids and leaf hoppers transmit a larger number of viral diseases and are most important .

- The viruses can be classified as:

  a) **Stylet borne viruses** (mostly aphid transmitted), which do not go into the system of insects and remain near the tip of the stylet and are lost after one or two visits. They are also called 'non-persistent viruses, e.g., *Cucumber mosaic virus, Papaya ring spot virus,* etc.

  b) **Circulative viruses,** mostly vectored by leaf hoppers, which take them into their system and after circulation, they are returned to the stylet, mixed with the saliva and are transmitted to healthy plants they visit, e.g., *Maize streak virus*

  - Some viruses of circulative nature may multiply inside the insect body and are called as '**propagative viruses'**. Leaf hoppers transmitted viruses are mostly circulative and propagative in nature, e.g., *Tomato spotted wilt virus*

  c) **Persistent viruses,** which are acquired by leaf hoppers and are incubated for 1-2 weeks and become viruliferous. Once they start transmitting viruses, they remain infective for long periods or even for rest of their life, e.g., *Banana bunchy top virus.*

  d) **Transovarial transmission,** in which the viruses once acquired are transferred to their following generations.

  Examples of other Insects transmitting viruses:

- Green peach aphid transmits *potato virus Y* (*Potyvirus*) and *potato leaf roll virus* (PLRV)
- Leaf hoppers transmit *rice stunt virus*, *aster yellow virus* etc.
- White flies can transmit *Papaya leaf curl virus*, *Yellow vein mosaic virus*, *Tomato leaf curl virus*
- Mealy bug transmits Swollen shoot of cocoa
- Thrips transmit *Tomato spotted wilt virus*
- Beetles transmit *Squash mosaic virus*, *Cowpea mosaic virus* and *Turnip yellow mosaic virus*

- Grass hopper transmits *Tobacco mosaic virus* (TMV), *Potato virus X* (PVX) and *Tobacco ring spot virus*.

Mite transmission

Mites transmitting viruses have piercing and sucking mouth-parts.

1. Examples are *Wheat streak mosaic virus*, *Peach mosaic virus* and viruses of sterility disease of pigeon pea.

Fungus transmission: Three major classes of fungi can transmit viruses.

(a) Chytridiomycetes- *Olpidium brassicae* transmits *Lettuce big vein virus*; and *Synchytrium endobioticum* transmits *Potato virus X* and *Potato mop top virus* .

(b) Plasmodiophoromycetes- *Polymyxa graminis* transmits *Wheat soil borne mosaic virus*, *Spongospora subterranea* transmits *Potato mop top virus*.

(c) Oomycetes- *Pythium ultimum* transmits *Pea false leaf roll virus*.

Nematode transmission:

2. **NEPO viruses-** *Longidorus* and *Xiphinema* species are shown to transmit several polyhedral viruses, such as *Grapevine fan leaf virus*, *Tobacco ring spot virus*, *Cherry leaf roll virus* and *Tomato black ring virus*.
3. **NETU viruses-** Species of *Trichodorus* and *Paratrichodorus* transmit tubular viruses, like *Pea early browning* and *tobacco rattle virus*.

Dodder transmission:

4. Plant viruses are also transmitted from one plant to another through the bridge formed between two plants by twining stems of the parasitic plant dodder (*Cuscuta sp.*)
5. Most commonly involved species are *C. campestris* transmitting *Cucumber mosaic virus*,*Tomato bushy stunt virus* and *Tobacco mosaic virus;* and *C. subinclusa* is known to transmit *Sugar beet curly top virus*.

Symptoms of viral diseases

- Viruses cause a number of symptoms on plants, like mosaic, mottle, vein clearing, vein banding, ring spots, enations, yellow vein mosaic, etc.

Nomenclature and Classification of Plant Viruses

- Traditionally, viruses are named after the most conspicuous symptom they produce on the first host. A virus causing mosaic on tobacco is called *Tobacco mosaic virus*, whereas the disease itself is called as tobacco mosaic.
- There have been frequent changes in the nomenclature and classification of viruses and generic names have been adopted.

- A **genus** is usually considered as a population of virus species that shares common characteristics and are different from other population of species.
- Currently 70 genera of plant viruses have been recognized.
- The genera are named either after the type species (*Caulimovirus* after *Cauliflower mosaic virus*) or are given a descriptive name often from a Greek or Italian word for a major feature of a genus, e.g., *Closterovirus* from the Greek word 'kloster' meaning 'a spindle or thread' – descriptive of virus particle shape; *Geminivirus* from the Latin word 'geminous' meaning twins to describe the particles.
- Secondly, genera are grouped together into family on common characteristics.
- There are 14 families recognized for plant viruses, such as *Reoviridae* and *Rhabdoviridae*, which are common with animal viruses. However, 22 genera have not yet been assigned any family and are called 'floating genera'.
- The family is either named after type member genus (e.g., *Caulimoviridae* named after Genus *Caulimovirus*) or given a descript to be named associated with genus for a major feature of family, e.g., *Geminiviridae* descriptive of virus particles.
- Only three orders have been accepted so far by International Committee for Taxonomy of Viruses (ICTV). The mononegavirales contains, among others the *Rhabdoviridae* in which there are two plant virus-families

Use of virus names

- The ICTV sets rules which are regularly revised on virus nomenclature and the orthography of taxonomic names.
- The last word of the species is 'virus'; and suffix word for a genus is 'virus', for a subfamily is 'virinae', for a family is 'viridae', for an order is 'virales'.
- **In formal taxonomic usage, the virus order, family, genus and species names are printed in italics or underlined with first letter being capitalized.**
- Other words in species names are not capitalized unless they are proper nouns or parts of proper noun.
- Also in formal usage, names of taxons should proceed the name being used e.g. Family *Caulimoviridae*, the Genus *Closterovirus*, the species *Potato virus Y*.
- However, in less formal instances which are widely used, the taxonomic unit is omitted.
- The plant viruses are classified on the basis of structure, physico-chemical properties, serological relationships, activities in the host plants and transmission.

Latest Classification

The plant viruses are classified in five major groups based on:

- Nature of the genome (RNA or DNA)
- Strandedness (single or double stranded)
- Method of replication

- Each group (not a recognized taxon) has orders, families, genera and species.
- The five groups are:

i) Single stranded positive sense RNA [(+) RNA] viruses
ii) Single stranded negative sense RNA [ss (-) RNA] viruses
iii) Double stranded RNA (ds RNA) viruses
iv) Double stranded DNA virus [ds DNA (RT)] viruses
v) Single stranded DNA [ss DNA] viruses

I. Single stranded positive sense RNA [(+) RNA] viruses:

- **Order: *Nidovirales***

i) Family: *Bromoviridae*, e.g., *Bromovirus* (*Brome mosaic virus*-BMV), *Alfamovirus (Alfalfa mosaic virus*-AMV), *Cucumovirus* (*Cucumber mosaic virus*-CMV) and *Ilarvirus* (*Tobacco streak virus*-TSV).

ii) Family: *Closteroviridae*, e.g., *Closterovirus* (*Beet yellows virus*-BYV), *Ampelovirus* (*Grapevine leaf roll associated virus* GLRaV).

iii) Family: *Comoviridae*, e.g., *Comovirus* (*Cowpea mosaic virus*), *Fabavirus* (*Broad bean wilt virus*), *Nepovirus* (*Nematode transmitted polyhedral virus*, like *Tobacco ring spot virus*).

iv) Family: *Flexiviridae*, e.g., *Potexvirus* (*Potato virus X*), *Carlavirus* (*Carnation latent virus*).

v) Family: *Luteoviridae*, e.g., *Luteovirus* (*Barley yellow dwarf virus*-BYDV) and *Polerovirus* (*Potato leaf roll virus*- PLRV)

vi) Family: *Potyviridae*,

This family is largest single group of plant viruses and has been studied more extensively. Members of genus *Potyvirus* are one of the most successful plant viral pathogens. e.g., *Potyvirus* (*Potato virus Y*-PVY), *Ipomovirus* (*Sweet potato mild mottle virus*-SPMMV) and *Bymovirus* (*Barley yellow mosaic virus*).

vii) Family: *Sequiviridae*, e.g., *Sequivirus* (*Parsnip yellow fleck virus*-PYFV) and *Waikavirus* (*Rice tungro spherical virus*- RTSV).

viii) Family: *Tombusviridae*, e.g., *Tombusvirus* (*Tomato bushy stunt virus*-TBSV), *Carmovirus* (*Carnation mottle virus*), *Necrovirus* (*Tobacco necrosis virus*-TNV)

ix) Family: *Tymoviridae*, e.g., *Tymovirus* (*Turnip yellow mosaic virus*- TYMV).

- Some of the very important viruses like *Tobamovirus (Tobacco mosaic virus*- TMV*)*, *Tobravirus* (*Tobacco rattle virus*-TRV), *Potexvirus* (*Potato virus X*-PVX) etc. have not been assigned any family yet.

II. **Single stranded negative sense RNA [ss (-) RNA] viruses:** Members of this group are only enveloped plant viruses.

Order: *Mononegavirales*

- **Family:** *Rhabdoviridae*, e.g., *Cytorhabdovirus* (*Lettuce necrotic yellows virus*- LNYV) and *Nucleorhabdovirus* (*Potato yellow dwarf virus*- PYDV).
- **Family:** Bunyaviridae, e.g., *Tospovirus* (*Tomato spotted wilt virus*-TSWV; *Groundnut bud necrosis virus*- GBNV)

III. **Double stranded RNA (ds RNA) viruses :** There is no order assigned.

- **Family:** *Rheoviridae*, e.g., *Fijivirus* (*Fiji disease virus*-FDV) and *Phytorheovirus* (*Wound tomur virus*- WTV).
- **Family:** *Partiviridae*, e.g., *Alphacryptovirus* (*White clover crypto*-virus *1*) and *Betacryptovirus* (*White clover crypto-virus 2*)

IV. **Double stranded DNA virus [ds DNA (RT) virus]:** No order has been assigned.

- **Family:** *Caulimoviridae*, e.g., *Caulimovirus* (*Cauliflower mosaic virus*- CaMV)

V. **Single stranded DNA [ss DNA] virus: No order has been assigned.**

- **Family:** *Geminiviridae*, e.g., *Mastrevirus* (*Maize streak virus*- MSV), *Curtovirus* (*Beet curly top virus*-BCTV), *Begomovirus* (*Bean golden mosaic virus*- BGMV), *Bhendi yellow vein mosaic virus*- BYMV and *Cassava latent virus*- CLV.

- **Family:** *Circoviridae*, e.g., *Nanovirus* (*Subterranean clover stunt virus; Banana bunchy top virus*-BBTV).

Lecture 12

ALGAE AND FLAGELLATE PROTOZOA CAUSING PLANT DISEASES

Aim: To acquaint the students with algae and flagellate protozoa causing plant diseases

Algae

- Parasitic algae are green in colour.
- ***Cephaleuros*** is the best known genus, and is a plant parasite living under leaf cuticle.
- It was first reported from India in the 19th century, causing damage to tea and coffee plantations.
- Now, over 400 hosts of *Cephaleuros* are recorded all over the world infecting hibiscus, orchids, euphorbias, citrus and forest trees, and 90 percent of its hosts are dicot.
- *Cephaleuros* belongs to phylum *Chlorophtya*, class *Ulvophyceae*, order *Trentepohliales*, and family *Trentepohliaceae*.
- There are 13 species of *Cephaleuros*, but 6 are more common. These are: *C. expansa, C. .henningsii, C. karstenii, C. minimus, C. parasiticus,* and *C. virescens.* Among these, *C. parasiticus* and *C. virescens* are most common and cause maximum damage.
- *C. virescens* causes ***red rust*** of tea and mango.

Red rust

- Fluffy, bright-orange red spots occur on leaves and stems that look very much like rust fungi.
- C. *virescens* has the misleading common name 'red rust'.
- Species of *Cephaleuros* have fungus-like filaments, sterile hairs and produce sporangiophores and zoosporangia on the lower surface of leaves that look like downy mildew fungi. Necrosis may be limited to the epidermis or spread into the deeper tissues of the leaves. Severe damage usually occurs on older leaves leading to defoliation.

Lichens

- Fungi parasitize *Cephaleuros* to form **lichens.**
- The lichenized state of *C. virescens* is identified as *Strigula elegans*.
- Early literature suggests that the fungus portion of the lichen (mycobiont) was responsible for plant damage.
- Recent findings show that the fungus parasitizes the alga, not the plant. Plant injury is caused by the alga much before a fungus colonizes it.

- Management of algal infections includes:

  i) Plant spacing and pruning to increase air circulation and light
  ii) Sanitation
  iii) Appropriate use of fertilizers and irrigation to promote plant growth.

Algal spot of magnolia

Lichen growing on apple tree trunk

Protozoa

- Certain protozoa, such as trypanosomatid flagellates belonging to class *Mastigophora*, order *Kinetoplastida*, family *Trypanosomatidae* are accepted as plant parasites even though Koch's postulates could not be established for them.
- However, evidence supporting their pathogenicity is more evident than that available for the fastidious bacteria and mollicutes, and so they are accepted as plant pathogens.
- The protozoa as such may be living freely, or living symbiotically or as parasites subsisting on organisms such as algae, yeasts, bacteria and other protozoa.
- Only the flagellates among the protozoa have been found to be associated with plant diseases.

Characteristics

- The flagellates have a long, oval or spherical body with a thin, flexible covering membrane or it may be armoured.
- They have one or more slender flagella, which are used for both locomotion and food capture.
- Many species of the flagellates like *Phytomonas* have been reported from several plants belonging to the family Euphorbiaceae (*P. davide* on *Euphorbia* sp.), Asclepiadaceae (*P. almassiani* on milkweed), Moraceae (*P. bancrofti* on *Ficus* sp.), and Rubiaceae (*P. leptovasorum* on coffee).

- Some other unknown forms are parasitic on oil palm and coconut palm.
- These parasites seem to be insect- transmitted, though an insect vector has been reported only for *P. almassiani*.
- Since most of those associated with laticiferous plants do not produce any clear symptoms, there is a feeling that these are just parasites but not pathogens.
- The non-laticiferous plants like coffee, coconut palm and oil palm develop characteristic external symptoms, such as leaf yellowing, wilting and malformation of phloem tissue often leading to considerable damage to the plant and ultimately death.

Phloem necrosis of coffee

- It is caused by *Phytomonas leptovasorum* and occurs in Suriname, British Guyana, and probably Brazil and Columbia.
- It affects the tree of *Coffea liberica* and *C. arabica*. Infected trees show sparse yellowing and dropping of leaves, and, only the young top leaves remain on the otherwise bare branches, followed by death of the trees, sometimes within 3-6 weeks.
- The flagellates can be traced from the roots upward to trunk, where they seem to migrate vertically in the phloem and laterally through the sieve plates into healthy sieve tubes.
- This disease can be transmitted through root grafts but not through green branch or leaf grafts. Its insect vector is a pentatomid *Lincus* sp.

Hart rot of coconut palms

- Hart rot has been known in Suriname since 1906, sometimes under the more appropriate names of lethal yellowing or bronze-leaf wilt , Cedros wilt and unknown disease.
- Many of the symptoms of hart rot are similar to those caused by lethal yellowing disease of coconut palm in the Caribbean, West Africa, Florida, the cause of the two diseases seem to be unrelated.
- The hart rot symptoms include yellowing and browning of the tips of the older leaves which subsequently spread to the younger leaves.
- Recently opened inflorescences are black , and unripe nuts of the symptomatic trees fall off.
- Flagellates of the genus *Phytomonas* occur in mature sieve elements of young leaves and inflorescences of hart rot affected coconut palms.
- The number and spread of the flagellates in the sieve tubes increase proportionally with the development of the disease.
- They are also transmitted by the pentatomid insects of genera *Lincus* and *Ochlerus.*

Sudden wilt (Marchitez) of oil palm

- The symptoms begin as browning of the tips of the lower leaflets of the oil palms.
- The browning subsequently spreads to the upper leaves and eventually becomes ash grey.
- In the mean time, root tips also begin to die and the whole root system deteriorates.
- As a result, the plant growth slows down, fruit bunches discolour and rot or fall off; and within a few weeks, all leaves become ash grey and dry up and the whole tree dies out.
- *Phytomonas* flagellates occur widely in the phloem sieve elements of the roots, leaves and inflorescences of the infected plants.
- These flagellates are also transmitted by Pentatomid insects *Lincus* and *Ochlerus*.

Empty root of Cassava

- The empty root disease was observed affecting certain cultivars of cassava (*Manihot esculenta*) in the Espirito Santo state of Brazil.
- Affected plants show poor root development.
- They remain small and slender, and contain little or no starch.
- Above ground parts of the infected plants show chlorosis and decline.
- Diseased plants contain numerous *Phytomonas* like protozoa in the laticiferous ducts but not in phloem.
- The empty root disease can be transmitted by grafting.
- It also spreads rapidly in the field, probably by the above insect vectors.

Lecture 13

FLOWERING PARASITIC PLANTS

Aim: To Acquaint the students with characteristics of flowering parasitic plants

Characteristics of Flowering Parasitic Plants

- The pathogenic flowering plants, also called parasitic angiosperms can be classified as root parasites or stem parasites.
- Root parasites (witchweed and broomrape) are more common and more diverse taxonomically.
- Stem parasites include the dodder (*Cuscuta*) and mistletoes (*Arceuthobium*).
- The angiospermic parasites can also be classified as holoparasites (total parasites) or hemiparasites (semiparasites).
- The holoparasites lack chlorophyll and are totally dependent on the host for nutrition. Thus, they are obligate parasites.
- The hemiparasites contain chlorophyll and make their own food, and absorb water and minerals from their host. But, in some cases, e.g., *Arceuthobium*, the photosynthesis is negligible and the parasite draws nutrition from the host. Practically, it is an obligate parasite.

Important Genera

- There are 277 genera and as many as 4100 parasitic plant species; but only 25 genera are recognized as plant pathogens.
- Out of these 25 genera, four are more damaging to crops viz., *Striga* (witcweed), *Orobanche* (broomrape), *Cuscuta* (dodder) and *Arceuthobium* (dwarf mistletoe).
- *Striga* is more prevalent in Asia and Africa, while *Orobanche* is worldwide, but more damaging in the Middle East.
- Both *Striga* and *Orobanche* produce microscopic seeds called “dust” seeds that persist in the soil for a long time, and are difficult to control.
- Dwarf mistletoes (*Arceuthobium* spp.) are the major pathogens of coniferous trees (belonging to families *Pinaceae* and *Cupressaceae).*
- *Dendrophthoe* (*Loranthus*) and *Viscum* species are parasitic on the forest, fruit and avenue trees; and are responsible for their die back and drying in Himachal Pradesh.

Root Parasites

***Striga* (whichweed)**

- *Striga* is an obligate root hemiparasite, although the seedlings above ground do form chlorophyll.
- Striga has made greater impact than any other parasitic angiosperm.
- It attacks important crops like maize, sorghum, pearl millet, rice, sugarcane and legumes (cowpea, groundnut, etc.).
- Two species, *S. asiatica* and *S. hermonthica* cause maximum damage to crops.
- *Striga* has a complex life cycle. It produces thousands of 'dust' seeds that are disseminated by wind and rain.
- The seeds after a dormant 'ripening' period of several months, respond to chemical signals exuded by the host.
- The chemical signals enable the *Striga* seeds to detect the type of host and its distance from the host.
- Seed germination of *Striga*, as in all obligate root parasites, is cryptocotyler i.e. the cotyledons remain within the seed when the radical comes out.
- The radical produces root hair like structures that glue it to the host.
- If the host is suitable, a haustorium is formed that penetrates and forms a link with the host vascular system.
- Once the parasite is established, the distinctive seedling of *Striga* is formed underground, which lacks chlorophyll, possesses scale-like leaves, and produces abundant adventitious roots that form additional haustoria, establishing more connections with the host.
- The seedlings exert great influence on the growth-regulating metabolism of the host, stimulating root production.
- Significant damage to the host occurs at this stage. The next stage is emergence of the seedlings above ground.
- Chlorophyll develops, and in due course, flower and seeds are formed. The life cycle is ready for a repeat.
- A major problem in control is persistence of the tiny seeds in the soil. Ethylene gas is introduced into the soil to induce seed germination, which becomes suicidal in absence of the host.
- Equipments and application methodologies have been developed to introduce the gas into the soil.

- Up to 90% seeds germinate by this method, and die in absence of the host.

Partial root parasite-*Striga*

Total root parasite-*Orobanche*

Orobanche (broomrape)

- This is an obligate root holoparasite, infecting legumes, solanaceous crops, carrot, cabbage, cauliflower, lettuce and sunflower.
- Total crop failure may occur in heavily infested soils. The parasite appears as whitish, yellowish or brownish stems, about 30 cm high that arise from the roots of infected host, and bear beautiful flowers, besides bracket-like leaves lacking chlorophyll.
- In general, *Orobanche* is a parasite of colder climate and need 10-20^{o}C of temperature for seed germination.
- This is the reason why it attacks tobacco during winter in India, but fails to infect sunflower during summer in the same field.
- Seed germination requirements of *Orobanche* are different from those of *Striga*.
- It needs low temperature (10-20^{o}C); the germinated seeds are geotropically neutral i.e. they do not grow downward in the soil and ethylene has no stimulatory effect.
- Its control is difficult due to the high longevity (more than 5 decades) of the seeds in the soil, their extremely small size (less than the thickness of human a hair), their production in extremely large number, and subterranean infection.

Stem Parasites

***Cuscuta* (dodder)**

- It is obligate stem holoparasite and is among the best known of all parasitic plants.
- Its slender, twining, orange-yellow, leaf less stems form conspicuous tangled mass on the host.
- The host range is large, though monocots are less preferred.
- Dodders are most important parasites of legumes.

- *Cuscuta campestris* is the most widely distributed among its 10 species that attack crops.
- It causes considerable damage to alfalfa, flax, sugarbeet, onion and other crops besides fruit, fodder and forest trees and shrubs . It also transmits viruses.
- The most effective means of control is seed sanitation. Several herbicides are effective on newly-germinated seeds.



Dodder (*Cuscuta* sp.) Showy mistletoe (*Dendrophthoe*) Leafy mistletoe (*Viscum* sp.)

Mistletoes

- Mistletoes are stem holoparasites occurring in three families of the order *Santalales* as follows:
- Family *Loranthaceae*: Showy mistletoes [*Loranthus* (*Dendrophthoe*)]
- Family *Santalaceae*: sandalwood (*Pyrularia*, *Santalum*)
- Family Viscaceae: Dwarf mistletoe (*Arceuthobium*), leafy mistletoe (*Viscum)*
- The showy "mistletoes" produce large and beautiful flowers that are pollinated by birds.
- The co-evolution of these parasites and the birds is also suggested by the seed dispersal mechanism operating in the birds.
- *Santalaceae*, the sandalwood family have a few members (*Pyrularia* etc.) that cause negative impact on their hosts.
- Family *Viscaceae* is called "Christmas mistletoe family", because their shoots with the white berries are used as door festoons during Christmas in temperate countries.
- The family has seven genera, and a large number (543) of species, most of which belong to three genera, *Viscum*, *Phoradendron* and *Arceuthobium.*
- The seeds are covered with a sticky substance, called 'viscin' that glues the seeds to the host surface.

***Arceuthobium* (Dwarf mistletoe)**

- *Arceuthobium* is the most important mistletoe in terms of economic losses, especially to the coniferous trees belonging to families *Pinaceae* and *Cupressacceae.*
- 11.3 million cubic meters of wood is lost annually due to the 'dwarf mistletoe' *Arceuthobium* in the US.
- It is a small (1.0-2.5 cm long) plant having green to brown aerial shoots, without secondary branching.
- Leaves are small, scale- like leaves. The major function of the aerial shoot is reproduction.
- The flowers are small and unisexual, present on same (monoecious) plants or on different (dioecious) plants.
- Pollination is brought about by insects and wind.
- Male aerial shoots are shed soon after pollination, but the female shoots persist until the seeds are formed and dispersed.
- Remnants of the aerial shoots persist as basal cups, on the host tree, where once the aerial shoots were formed.
- The seeds, which are discharged explosively from the fruit at the rate of 27 metres per second, reach up to 16 metres.
- The seed sticks to the surface by the viscin coating.
- It slides to the base of the "needle" where it germinates.
- The radicle forms a hold fast from which the haustorium emerges and penetrates the host tissue.
- Thus endophyte is formed, but it takes one year to form the aerial shoots, and 3-10 years to complete the life cycle (infection to seed dispersal).
- This long life cycle is profitably used in disease management.
- Selective removal of infected trees has been highly effective in controlling *Arceuthobium* infestation.
- Chemical control has also been successful with ethephon, an environmentally safe chemical.

Lecture 14

NON-PARASITIC CAUSES OF PLANT DISEASES

Aim: To acquaint the students with non-parasitic causes of plant diseases

- Diseases caused by non- parasitic/ abiotic (nonliving) agents are not transmitted from one plant to another. Thus, they are not infectious, and also called non-infectious diseases or simply, disorders.
- Extremes of temperature and water (flooding or drought), deficiency or excess of essential nutrients, presence of toxic chemicals in air, water and soil, transplant shock and mechanical injury are the important causes of abiotic diseases.
- Surprisingly, the symptoms of non-infectious (non-parasitic) diseases resemble those produced by living agents viz., fungi, bacteria, viruses and nematodes. If no signs of these organisms are present, a nonliving agent may be the cause of the disease.
- Meteorological weather reports and plant and soil analyses for mineral elements is the next step to confirm the disease agent.

Temperature Extremes

- Sudden rise and fall in temperature causes injury to plants.
- Harmful effects of chilling, freezing and sunburn are well known.
- The plants may get adapted to their climate and show chill or frost resistance.

Chilling injury: It occurs at temperature close to 0^{o}C. Tropical plants begin to experience cold damage at 5-10^{0}C.

- Symptoms include wilting of the upper portions of stems and leaves, blackening or softening of the plant tissue, surface pitting, necrosis or failure of ripening of fruits.
- This injury is severe in some warm season fruits.

Freezing or Frost injury: It occurs at temperatures below 0^{o}C.

- It is caused by formation of ice. Since water in the intercellular spaces is pure, ice crystals are first formed there, then inside the cells.
- The crystals formed inside the cells damage the cell organelles.

High temperature and dry winds: They cause rapid loss of water.

- Leaf margins turn yellow or brown and leaves fall off prematurely.
- Sun-scald injury occurs when shade loving plants are suddenly exposed to direct sun.
- Sunken brown areas on apples, water-soaked areas on tomatoes are examples of heat injury.
- High temperature also causes water core symptoms in apple.

Frost injury in banana **Water core of apple**

Soil Moisture Extremes

Low soil moisture, which occurs during drought, causes accumulation of toxic ions of manganese and boron, which damage tissues and cause stomatal closure.

- This adversely affects the plants. Wilting discoloured foliage, twig and branch 'die back' in the crown, and death of fine roots are the symptoms of water deficiency.

Excess soil moisture or flooding results in diminished oxygen supply in the soil water that kills the root.

- Symptoms of oxygen deficiency, during high moisture are reduced growth, small leaves and thin crowns, twig and branch 'die back' and plant death.

Unfavourable light

Insufficient light causes etiolation, stunted growth, and reduction in flowering.

High light intensity leads to scorching and rolling of leaves and drying of flowers.

Enhanced photoperiod results in abnormal shape, erratic flowering etc.

Lack of Oxygen

- Apart from the asphyxiation of plant roots in waterlogged soils and its adverse effects on the plants as discussed above, lack of oxygen may also result from its failure to diffuse adequately both between and within fruits or the storage organs kept in bulky piles or under poor ventilation.
- In such stress conditions, the cells die of suboxidation and results in storage diseases such as **black heart of potatoes**, in which the cortex of the affected tuber is blackened; and **internal browning in apple**.

Mineral Nutrient Imbalances

- Currently seventeen elements (nickel is the latest addition) are recognized as essential for plant growth. Among them, thirteen are found in soil mineral.

- The essential elements are divided into three categories: **macronutrient** elements, **micronutrient** elements (or trace elements) and **beneficial** elements.
- Carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium and sulphur are macronutrients.
- The micronutrients (needed less than 100µg per gram in dry tissue) include iron, manganese, zinc, copper (these are metal micronutrients) boron, molybdenum, chlorine and nickel.
- Beneficial elements, although not essential, do provide benefit to the plant.

Mineral deficiencies

- When the soil is deficient in any element, or the element is 'unavailable' due to, for example unsuitable pH, it shows up as typical symptoms in the plant.
- The common symptoms of nutrient deficiencies are: **reduced growth, leaf chlorosis of different patterns, and necrosis**.
- Symptoms are very similar and occasionally indistinguishable for different elements.
- Soil and plant tissue analyses are therefore, necessary for the symptoms. The deficiency may be **acute or chronic**. The former is due to sudden unavailability of the element, while he latter is a continuous insufficient supply.
- Nutrient mobility plays an important role in determining the site of the deficiency symptoms.
- For **highly mobile elements** like nitrogen and potassium, the deficiency symptoms appear predominantly in older and mature leaves.

- Deficiency of **poorly mobile elements**, like calcium, boron and iron show up in younger leaves, opposite of what happens with highly mobile elements.

Bitter pit of apple due to Ca deficiency

Spray injury on pear fruits

- The symptoms of deficiency of **moderately mobile elements** like magnesium and sulphur, are uniformly spread all over the plant.

- The symptoms of mineral deficiencies is recorded in the following table:

Sr.No	**Deficient element**	**Symptoms**
1	Nitrogen	Poor growth, chlorosis of leaves, delayed flowering and fruiting
2	Phosphorus	Poor growth, blue/green, but not yellow, colour of leaves, oldest leaves affected first
3	Potassium	Brown scorching and curling of leaf tip, yellowing of leaf veins
4	Calcium	Curling and tip burn of leaves, stunted growth abnormal development of meristmatic tissues, and eventual death of buds and root tips. Also causes **bitter pit in apple**.
5	Magnesium	Interveinal chlorosis, giving a mottled appearance to leaves, similar to virus infection
6	Sulfur	Chlorotic leaves, petioles and veins distinctly red.
7	Iron	Chlorosis of leaves, young leaves appear bleached, symptoms similar to manganese deficiency
8	Manganese	Chlorotic leaves with green veins
9	Zinc	Younger leaves yellow, pitting on lower leaf surface, leaves and internodes shortened, giving a rosette-like look to plants; guttation increased
10	Copper	Leaves pale and curled; petioles droop down
11	Boron	Drying of growing tip, bushy stunted growth, and internal tissue breakdown.
12	Molybdenum	Chlorosis of leaves; symptoms similar to nitrogen deficiency. Also causes **whiptail disease in cauliflower**.
13	Chloride	Leaves of abnormal shapes, and with interveinal chlorosis.

Aluminum toxicity

- Aluminum is one of the most abundant element is the earth crust.
- It is toxic to several plants at 2-3 ppm concentration, when the soil pH is 5.5 and above.

Symptoms

- Symptoms of aluminum toxicity are not easily identifiable.
- The foliar symptoms resemble those of phosphorus deficiency, viz., overall stunting, blue/green colour of leaves.

- In some cases, aluminum toxicity appears as an induced calcium deficiency, showing curling, rolling and tip burn of young leaves, and collapse of growing points or petioles.

Injury By Air Pollution (SO_2, F, Cl_2, O_3, PAN,C_2H_4)

- Injury caused by air pollution is often evident on plants before it affects human beings and other animals.
- Air pollutants may be inorganic (sulphur dioxide-SO_2, fluorine-F , chlorine-Cl_2, and ozone-O_3; or organic (peroxy-acetyl nitrate- PAN, and ethylene- C_2H_4).
- The symptoms caused by air pollutants are very similar to those caused by biotic and other abiotic factors, like mineral deficiency and excesses, or the adverse effects of temperature, water and light.
- In general, the visible symptoms are of three types:

  i) Collapse of leaf tissues, forming necrotic symptoms
  ii) Yellowing or other colour changes
  iii) Alterations in growth
  iv) Premature defoliation

- Damage is severe during warm, still and humid weather, when the atmospheric pressure is high.
- In this condition the pollutants accumulate near the earth's surface, as the warm air above in the atmosphere traps the cooler air near the ground. This is called **air inversion**.
- The plants vary in their response to the pollutants, and are categorized into very sensitive and somewhat resistant plants for each type of pollutant.
- For control and disease management, resistant plants should be grown in SO_2 prone areas, and plant vigor should be maintained.
- In good health, plants resist all types of injury better than weakened plants.
- The damage to plants by air pollutants is given in following Table.

Symptoms caused by air pollutants

Air pollutant	Symptoms
Sulphur dioxide	Marginal and inter-veinal chlorosis, reduced growth and defoliation. Near brick kilns, SO_2 toxicity is known to **cause black tip or tip necrosis** of mango in India.
Fluoride	Yellowish mottle to scorching at margins and the tips of broad-leaf plants, tip burns in grasses and conifers
Chloride	Marginal and inter-veinal chlorosis, similar to that caused by sulphur dioxide and chloride.

Ozone	Chlorosis and necrosis, flecking, bronzing and reddening of leaves, stunted growth, reduction in yield more pronounced in dicots (soybean, cotton and peanut) than in monocots (sorghum, corn and wheat)
Organic	
PAN	Collapse and death of leaf tissues, typical leaf glazing, browning or silvering; stunted growth, early senescence and defoliation
Ethylene	Modifies activity of other hormones, affecting normal organ development, shoot and leaf epinasty, accompanied by stunted growth.

Lecture 15

INFECTION PROCESS

Aim: To acquaint the students with the infection process

- In every infectious disease, a series of more or less distinct events occurs in succession which leads to the development of the disease. This chain of events is called **Pathogenesis** or disease cycle.
- A **disease cycle** sometimes corresponds fairly closely to the life cycle of the pathogen, and refers primarily to the appearance, development and perpetuation of the disease as a function of the pathogen rather than to the pathogen itself.
- The **life cycle** of a pathogen refers to the stage or successive stages in the growth and development of the pathogen (or any organism) that occurs between the appearance and reappearance of the same stage (e.g. spore) of the organism.
- Disease cycle involves changes in the plant and its symptoms as well as those in the pathogen; and spans periods with in a growing season and from one growing season to the other.

Primary Events in the Disease Cycle

i) Inoculation

ii) Penetration

iii) Establishment of the infection

iv) Colonization (invasion)

v) Growth and reproduction of the pathogen

vi) Dissemination of the pathogen

vii) Survival of the pathogen in the absence of the host i.e. overwintering and over-summering (over-seasoning) of the pathogen

- In some cases, there may be several infection cycles within one disease cycle.

Inoculum and Inoculation

- **Inoculum**- The infective pathogen propagules coming in contact with the host constitutes the inoculum.
- **Inoculation** is the initial contact of the pathogen with the site of the plant where infection is possible.

- In fungi, the inoculum may include the spores, sclerotia (compact mass of mycelium) or fragments of the mycelium.
- In bacteria, phytoplasmas, protozoa, viruses and viroids, the inoculum is always their whole individuals.
- In nematodes, it may be adult nematodes, juveniles or eggs. In parasitic flowering plants, the inoculum may be plant fragments or seeds.
- **Primary inoculum -**The inoculum that survives dormant in the winter or summer and brings about original infections in the spring and autumn is called **primary inoculum,** and the infections it causes are called **primary infections**.
- **Secondary inoculum-** The inoculum produced from primary infections is called **secondary inoculums,** and it, in turn causes **secondary infections**.
- Generally, the amount of inoculum and prevalence of favourable environmental conditions determine the success of infection.
- **Inoculum potential** has been defined as the 'energy of growth of parasite available for infection of host at the surface of the host organ to be infected'.
- It is the resultant of action of the environment, the vigour of the pathogen to establish an infection, the susceptibility of the host and amount of the inoculum present.
- The inoculum, which survives whether on perennial plants, plant debris or soil, or on the propagative planting material is carried to the host plants mostly by wind, water, insects or man.
- Only a tiny fraction of the potential inoculum produced actually lands on the susceptible host plants.
- The bulk of the produced inoculum lands on the things that cannot become infected.
- Some types of inoculum, in the soil, e.g., zoospores and nematodes may be attracted to the host plant by chemical substances like sugars and amino acids diffusing out of the plant roots. This process is known as **chemotaxis**.
- Vector transmitted pathogens are usually carried to their host plants with an extremely high efficiency.

Pre-penetration Activities of the Pathogen on the Host Surface

- Almost all fungi, bacteria and parasitic higher plants, must be first attached to the host surfaces.
- This attachment takes place through their adhesive materials which are composed of water insoluble polysaccharides, glycoproteins, lipids and fibrillar materials, which when moistened become sticky and help the pathogens to adhere to the plant.

- Pathogens, such as phytoplasmas, fastidious bacteria, protozoa and most viruses are placed directly into the cells of the plants by their vectors.
- Many fungal pathogens first grow on the surface of the host to get proper mechanical, morphological and chemical strength to bring out the penetration of the barriers.
- In *Rhizoctonia solani*, the fungus first forms infection cushions and appressoria and from their multiple infections take place by means of infection pegs.
- In *Armillariella mellea*, the fungus hyphae form the **rhizomorphs** (aggregation of hyphae forming rope like/root like structures), which produce desired amount of enzymes required for direct penetration of the host.
- In other fungi, the spores landed on the host surfaces germinate producing germ tubes, which cause penetration, directly or indirectly or they first produce **appressoria** from which infection threads develop and penetrate the host.
- **Appressoria** are swollen structures formed on the tips of the germ tubes and facilitate in attachment and penetration of the host, which are produced by their thigmotropic (contact) response to the hard surfaces, and in turn produce infection hyphae or infection pegs and exert pressure to affect the direct penetration of the host.
- Seeds of parasitic flowering plants germinate by producing a radical which either penetrate the host plant directly or first produces a small plant that subsequently penetrates the host plant by means of specialized feeding structures called **haustoria**.
- Nematode eggs also require conditions of favourable temperature and moisture to become activated and hatch.

Penetration

Plant pathogens penetrate the plant surfaces by direct penetration of the cell walls, natural openings or through wounds.

Direct penetration

- Direct penetration through intact plant surfaces is probably the most common type of penetration by fungi, oomycetes and nematodes and only type of penetration through parasitic flowering plants. None of the other pathogens can enter plants by direct penetration.
- Hemibiotrophs or non-obligate parasitic fungi do so through a fine hyphae produced directly by the spore or the mycelium.
- The obligate parasites do so through a penetration peg produced by an appressorium.
- They are formed at the point of contact of the germ tube or mycelium with a plant surface.

- The fine hyphae growing towards the plant surface pierces the cuticle and the cell wall through mechanical force and enzymatic softening of the cell wall substances.
- Most fungi form an appressorium at the end of germ tube, it being bulbous or cylindrical with a flat surface in contact with the surface of the host plant.
- Then a penetration peg grows from the flat surface of the appressorium towards the host and pierces the cuticle and cell wall.
- The penetration peg grows into the small hyphae generally much smaller in diameter than the normal hyphae of the fungus and regains its normal diameter once inside the cell.
- Parasitic higher plants also form an appressorium and penetration peg and the point of contact of the radical with the host plant; and penetration is similar to that in fungi.
- Direct penetration in nematodes is accomplished by repeated back and forth thrusts of their stylets. Such thrusts finally create fine opening in the cell wall. It then inserts its stylet into the cell so the entire nematode enters the cell.

Indirect penetration (through wounds)

- All bacteria, most fungi, some viruses and all viroids can enter through various kinds of wounds.
- Some viruses and all mollicutes, fastidious vascular bacteria and protozoa enter plants through wounds made by their vectors.
- The bacteria and fungi may grow briefly on the lacerated or dead tissues before they advance in to the healthy tissues.
- The penetration of viruses, phytoplasmas, fastidious bacteria and protozoa through wounds depends on their deposition by the vector on fresh wounds created at time of inoculation.
- Some viruses and viroids penetrate through wounds made by human hands and tools.

Indirect penetration through natural openings

Stomata are natural openings and are more in number on lower leaf surfaces; measuring about 10-20 x 5-8 µm, and are open in the day time; but are more or less closed at night.

- Bacteria which are present in a film of water over stomatal openings, swim through it easily and reach the sub-stomatal cavity when they can multiply and start infection.
- Fungal spores generally germinate on the plant surface and germ tubes may grow through stomata.
- The germ tubes form an appressorium that fits tightly over one stomata and normally one fine hypha grows from it into the stoma.

- It enlarges through sub-stomatal cavity giving rise to several small hyphae that actually invade the cells of the host plants directly or through hasutoria.
- Although some fungi can penetrate through closed stomata, others penetrate stomata only while they are open, e.g., *Puccinia graminis tritici,* the cause of stem rust of wheat.

Hydathodes are more or less permanently open pores at the margins and tips of the leaves. They are connected to the veins and secrete droplets of liquids called guttation drops containing various nutrients.

- Some bacteria, e.g., the one which causes black rot of cabbage (*Xanthomonas campestris* pv. *campestris*) use these pores as means of entry into the leaves.
- *Erwinia amylovora* causing fire blight of apple and pear also enter blossoms through the **nectarthodes** or **nectaries** which are similar to hydathodes but are present on the receptacle or other parts of the flower.

Lenticels are openings on the fruits, stems and tubers that are filled with loosely connected cells to allow passage of air and seem to offer little resistance to pathogen entry.

- Lenticel and wound penetration are quite similar. Many lenticel invaders can also enter through wounds, particularly soil borne pathogens like *Streptomyces scabies* (causing potato common scab), *Erwinia carotovora* (soft rot of vegetables), *Armillariella mellea* (root rot), *Spongospora subterranea* (potato powdery scab), , *Penicillium expansum* (causing blue mould rot), *Monilinia fructicola* (causing brown rot of apple) and *Nectria galligena* (causing apple canker).
- The germ tubes or hyphae of invading fungi grow between the lenticel cells and enter the plant tissues.

Infection

Infection is the process by which pathogens establish contact with susceptible cells or tissues of the host and obtain nutrients from the tissues.

- Successful infection results in the appearance of symptoms, viz., discolouration, malformation or necrotization of the affected plant parts.
- Some infections do not produce symptoms right away, and remain **latent** but do so at a later time when environmental conditions and/or stage of plant become more favourable.
- The time interval between inoculation and the appearance of disease symptoms is called **incubation period**.
- It varies from few days to years with different pathogens-host combinations, stage of host and prevailing environmental conditions.

- During infection, some pathogens obtain nutrition from the living cells, often without killing them or at least not for a long time; others kill the cells and utilize their contents as they invade them; and still others kill cells and disorganize the surrounding tissues.
- During infection, pathogens release a number of biologically active substances (e.g., enzymes, toxins and growth regulators) that may affect the structural integrity of the host cells or their physiological processes.
- In response, the host reacts with a variety of defence mechanisms.
- For a successful infection, the host must be susceptible, the pathogen must be virulent and the environment must be favourable.
- When these conditions occurred at an optimum, the pathogen can further invade the host plant up to the maximum of its potential even in the presence of plant defenses; and in consequence the disease develops.

Invasion

Various pathogens invade hosts in different ways and to different extents.

- Some fungi, such as those causing powdery mildews produce mycelium only on the surface of the plants and send **haustoria** into the epidermal cells.
- Others such as those causing apple scab and black spot of rose produce mycelium that grows only in the area between the cuticle and epidermis showing **sub-cuticular growth**.
- Most fungi spread into the tissue of the plant organs either by growing directly through the cells as **intracellular mycelium** or by growing between the cells as **intercellular mycelium.**
- Fungi that cause vascular wilts invade the **xylem vessels** of the plants.
- Bacteria invade tissues inter-cellularly, although when parts the cell walls dissolve, they also grow intra-cellularly.
- Bacteria causing vascular wilts and fastidious bacteria (*Xylella fastidiosa*) invade the xylem vessels.
- Viruses and viroids invade all types of living cells, phytoplasma and protozoa invade phloem sieve tubes and a few adjacent phloem parenchymatous cells.
- **Local infections-** Many infections caused by fungi, bacteria, nematodes, viruses and parasitic flowering plants are local, i.e., they involve a single cell, a few cells or a small area of the plant. They may remain localized throughout the growing season or may enlarge slightly or very slowly.
- **Systemic infections-** Other infections enlarge more or less rapidly and may involve an entire plant organ, a large part of plant or even the entire plant.

- Infections caused by fastidious bacteria, phytoplasma, protozoa, and natural infections caused by viruses and viroids are **systemic,** i.e., the pathogen from one initial point spreads and invades most or all susceptible cells and tissues throughout the plant. For example, vascular wilts, some downy mildews, white rust of crucifers, loose smut and hill bunt of wheat.

Growth and reproduction (colonization) of the pathogen

- After infection, pathogens grow, multiply or both within the plant tissues and invade and colonize the plant to a lesser or greater extent.
- Growth and reproduction of the pathogen (also called colonization) in or on the infectious tissues are actually two **concurrent sub-stages of disease development**.
- Most of the fungi and parasitic higher plants generally invade and infect the plant tissues by growing on or into them.
- They continue to grow and branch out within the infected host and spread into more and more of the plant until, its spread is stopped or the plant is dead.
- All other pathogens, namely, bacteria, phytoplasmas, viruses, viroids and protozoa do not increase in size much if at all, as their size and shape remains relatively unchanged throughout their existence.
- They invade and infect the new tissues within the plants by reproducing at a rapid rate and increasing their number tremendously in the infected tissues.
- Their progeny may then be carried passively into the new cells and tissues through plasmodesmata (some viruses, viroids), phloem (viruses, viroids, phytoplasmas, some fastidious bacteria, protozoa) or xylem (some bacteria).
- Alternatively, as happens with bacteria they may move through cells on their own power.
- **Plant pathogens reproduce in a variety of ways**.

i) Fungi reproduce by means of **spores**, either asexually or sexually.

ii) Parasitic higher plants produce **seeds**.

iii) Bacteria and phytoplasma reproduce by **fission** in which one mature individual splits into two equal, smaller individuals.

iv) Viruses and viroids are **replicated** inside the host cells.

Lecture 16

ROLE OF ENZYMES AND TOXINS IN PLANT DISEASE DEVELOPMENT

Aim: To acquaint the students with role of enzymes and toxins in plant disease development

Enzymes

- **Enzymes** are generally large protein molecules that catalyze organic reactions in living cells and in solutions.
- Because most kinds of chemical reactions that occur in a cell are enzymatic, there are almost as many kinds of enzymes as there are chemical reactions.
- Each enzyme, being a protein, is coded for by a specific gene.
- Some enzymes are present in cells at all times (constitutive).
- Many are produced only when they are needed by the cell in response to internal or external gene activators (induced).
- Each type of enzyme often exists in several forms known as isozymes that carry out the same function but may vary from one another in several properties, requirements, and mechanism of action.

Plant Substances and Their Enzymatic Degradation

- Aerial plant part surfaces consist primarily of cuticle and/or cellulose, whereas root cell wall surfaces consist only of cellulose.
- Cuticle consists of cutin, wax and covering of layer of wax
- The lower part of cutin contains pectin, cellulose lamellae and a thin layer of pectic substances; below that there is a layer of cellulose.

Cuticular wax

- Plant waxes are found as granular, blade, or rod-like projections or as continuous layers outside or within the cuticle of many aerial plant parts.
- The presence and condition of waxes at the leaf surface affect the degree of colonization of leaves and the effect varies with the plant species.
- Electron microscope studies suggest that several pathogens, e.g., *Puccinia hordei*, produce enzymes that can degrade waxes.

Cutin

- Main component of the cuticle.

- The upper part is admixed with waxes.
- The lower part is admixed with pectin and cellulose.
- Cutin is insoluble polyester of 16- and 18-carbon hydroxy fatty acids.
- Many fungi and a few bacteria produce cutinases and/or nonspecific esterases, i.e., enzymes that can degrade cutin.
- Cutinases break cutin molecules and release monomers (single molecules) as well as oligomers (small groups of molecules) of the component fatty acid derivatives from the insoluble cutin polymer.

Pectic substances

- Main components of the middle lamella, i.e., the intercellular cement that holds in place the cells of plant tissues.
- Also make up a large portion of the primary cell wall in which they form an amorphous gel filling the spaces between the cellulose microfibrils.
- Are polysaccharides consisting mostly of chains of galacturonan molecules interspersed with a much smaller number of rhamnose molecules and small side chains of galacturonan, xylan and some other five carbon sugars.

Degradation of pectic substances

- Several enzymes degrade pectic substances and are known as **pectinases** or **pectolytic enzymes**.
- The **pectin methyl esterases** remove small branches off the pectin chains.
- Pectin methyl esterases have no effect on the overall chain length, but they alter the solubility of the pectins and affect the rate at which they can be attacked by the chain-splitting pectinases.
- Pectinases cleave the pectic chain and release shorter chain portions containing one or a few molecules of galacturonan.
- Some chain splitting pectinases, called **polygalacturonases**, split the pectic chain by adding a molecule of water and breaking (hydrolyzing) the linkage between two galacturonan molecules.
- Others, known as **pectin lyases**, split the chain by removing a molecule of water from the linkage, thereby breaking it and releasing products with an unsaturated double bond.

Cellulose

- Cellulose - a polysaccharide, but consists of chains of glucose (1–4) β-d-glucan molecules.

- The glucose is produced by a series of enzymatic reactions carried out by several cellulases and other enzymes.
- One cellulase (C1) attacks native cellulose by cleaving cross-linkages between chains.
- A second cellulase (C2) also attacks native cellulose and breaks it into shorter chains.
- These are then attacked by a third group of cellulases (Cx), which degrade them to the disaccharide cellobiose.
- Finally, cellobiose is degraded by the enzyme β -glucosidase into glucose.

Cross-linking glycans (hemicelluloses)

- The enzymatic breakdown of hemicelluloses requires the activity of many enzymes.
- Several hemicellulases seem to be produced by many plant pathogenic fungi.
- Depending on the monomer released from the polymer on which they act, the particular enzymes are called xylanase, galactanase, glucanase, arabinase, mannase, and so on.
- The non-enzymatic breakdown of hemicelluloses by activated oxygen, hydroxyl, and other radicals produced by attacking fungi also occurs.

Suberin

- Suberin is found in certain tissues of various underground organs, such as roots, tubers, and stolons, and in periderm layers, such as cork and bark tissues.
- Suberins are also formed in response to wounding and to pathogen-induced defenses of certain organs and cell types.
- Although plants obviously produce enzymes that synthesize suberin, it is not known whether or how pathogens break it down during infection.

Lignin

- Found in the middle lamella, as well as in the secondary cell wall of xylem vessels and the fibres that strengthen plants.
- Also found in epidermal and occasionally hypodermal cell walls of some plants.
- The lignin content of mature woody plants varies from 15 to 38% and is second only to cellulose in abundance.
- Lignin is an amorphous, three-dimensional polymer that is different from both carbohydrates and proteins in composition and properties.
- It is obvious that enormous amounts of lignin are degraded by microorganisms in nature, as is evidenced by the yearly decomposition of all annual plants and a large portion of perennial plants.

Cell wall flavonoids

- Flavonoids are a large class of phenolic compounds that occur in most plant tissues and, especially, in the vacuoles.
- Also occur as mixtures of single and polymeric components in various barks and heartwoods.
- Among the various functions of flavonoids, some act as signaling molecules for certain functions in specific plant-microbe combinations.

Cell wall structural proteins

- Cell walls consist primarily of polysaccharides, i.e., cellulose fibres embedded in a matrix of hemicelluloses and pectin, but structural proteins, in the form of glycoproteins, may also form networks in the cell wall.
- Four classes of structural proteins have been found in cell walls.
    - **hydroxyproline-rich glycoproteins (HRGPs)**
    - **proline-rich proteins (PRPs)**
    - **glycine-rich proteins (GRPs)**
    - **arabino-galactan proteins (AGPs)**

Enzymatic degradation of substances contained in plant cells

- Most kinds of pathogens spend all or part of their lives in association with or inside the living protoplast.
- These pathogens obviously derive nutrients from the protoplast.
- The great majority of fungi and bacteria -obtain nutrients from protoplasts after the latter have been killed.
- Some of the nutrients, e.g., sugars and amino acids are sufficiently small molecules to be absorbed by the pathogen directly.
- Some of the other plant cell constituents, however, such as starch, proteins and fats can be utilized only after degradation by enzymes secreted by the pathogens.

Proteins

- Plant cells contain innumerable different proteins, which play diverse roles as catalysts of cellular reactions (enzymes) or as structural material (in membranes and cell walls).
- Proteins are formed by the joining together of numerous molecules of about 20 different kinds of amino acids.

- All pathogens seem to be capable of degrading many kinds of protein molecules.
- The plant pathogenic enzymes involved in protein degradation are similar to those present in higher plants and animals and are called **proteases** or **proteinases** or, occasionally, peptidases.

Starch

- Starch is a glucose polymer and exists in two forms:
    - amylose, an essentially linear molecule
    - amylopectin, a highly branched molecule of various chain lengths
- Most pathogens utilize starch and other reserve polysaccharides in their metabolic activities.
- The degradation of starch is brought about by the action of enzymes called **amylases**.
- The end product of starch breakdown is glucose and is used by the pathogens directly.

Lipids

- Various types of lipids occur in all plant cells, with the most important being **phospholipids** and **glycolipids**, both of which, along with proteins, are the main constituents of all plant cell membranes.
- The latter form a hydrophobic barrier that is critical to life by separating cells from their surroundings and keeping organelles such as chloroplasts and mitochondria intact and separate from the cytoplasm.
- **Oils** and **fats** are found in many cells, especially in seeds where they function as energy storage compound.
- The common characteristic of all lipids is that they contain fatty acids, which may be saturated or unsaturated.
- Several fungi, bacteria, and nematodes are known to be capable of degrading lipids. Lipolytic enzymes, called **lipases**, **phospholipases**, and so on, hydrolyze the lipid molecules with liberation of the fatty acids.

Microbial Toxins in Plant Disease

- Toxins are extremely poisonous substances and are effective in very low concentrations.
- Fungi and bacteria may produce toxins in infected plants as well as in culture medium.
- Toxins injure host cells either by affecting the permeability of the cell membrane or by inactivating or inhibiting enzymes and subsequently interrupting the corresponding enzymatic reactions.

- Certain toxins act as antimetabolites and induce a deficiency for an essential growth factor.

Non-host specific or **non-host selective toxins**

- Several toxic substances produced by phytopathogenic microorganisms have been shown to produce all or part of the disease syndrome not only on the host plant, but also on other species of plants that are not normally attacked by the pathogen in nature. Such toxins, called **non-host-specific** or **non-host-selective** toxins.

Tabtoxin

- Tabtoxin is produced by the bacterium *Pseudomonas syringae* pv. *tabaci*, which causes the wildfire disease of tobacco.
- Tabtoxin is a dipeptide composed of the common amino acid threonine and the previously unknown amino acid tabtoxinine.
- Tabtoxin as such is not toxic, but in the cell it becomes hydrolyzed and releases tabtoxinine, which is the active toxin.
- Toxin-producing strains cause necrotic spots on leaves, with each spot surrounded by a yellow halo.
- Sterile culture filtrates of the organism, as well as purified toxin, produce symptoms identical to those characteristic of wildfire of tobacco not only on tobacco, but in a large number of plant species belonging to many different families.
- Strains of *P. syringae* pv. *tabaci* sometimes produce mutants that have lost the ability to produce the toxin.

Phaseolotoxin

- Phaseolotoxin is produced by the bacterium *Pseudomonas syringae* pv. *phaseolicola*, the cause of halo blight of bean and some other legumes.
- Phaseolotoxin is a modified ornithine–alanine–arginine tripeptide carrying a phosphosulfinyl group.
- Soon after the tripeptide is secreted by the bacterium into the plant, plant enzymes cleave the peptide bonds and release alanine, arginine, and phosphosulfinylornithine, which is the biologically functional moiety of phaseolotoxin.
- The localized and systemic chlorotic symptoms produced in infected plants are identical to those produced on plants treated with the toxin alone so they are apparently the results of the toxin produced by the bacteria.

- Infected plants and plants treated with purified toxin also show reduced growth of newly expanding leaves, disruption of apical dominance, and accumulation of the amino acid ornithine.
- Phaseolotoxin plays a major role in the virulence of the pathogen by interfering with or breaking the disease resistance of the host toward not only the halo blight bacterium, but also several other fungal, bacterial, and viral pathogens.

Tentoxin

- Tentoxin is produced by the fungus *Alternaria alternata* (previously called *A. tenuis*), which causes spots and chlorosis in plants of many species.
- Tentoxin is a cyclic tetrapeptide that binds to and inactivates a protein (chloroplast-coupling factor) involved in energy transfer into chloroplasts.
- The toxin also inhibits the light-dependent phosphorylation of ADP to ATP. In sensitive species, tentoxin interferes with normal chloroplast development and results in chlorosis by disrupting chlorophyll synthesis.
- An additional but apparently unrelated effect of tentoxin on sensitive plants is that it inhibits the activity of polyphenol oxidases, enzymes involved in several resistance mechanisms of plants.
- Both effects of the toxin, namely stressing the host plant with events that lead to chlorosis and suppressing host resistance mechanisms, tend to enhance the virulence of the pathogen.

Cercosporin

- Cercosporin is produced by the fungus *Cercospora* and by several other fungi.
- It causes damaging leaf spot and blight diseases of many crop plants, such as Cercospora leaf spot of zinnia and grey leaf spot of corn.
- Cercosporin is unique among fungal toxins in that it is activated by light and becomes toxic to plants by generating activated species of oxygen, particularly single oxygen.

Other non-host-specific toxins

- Fumaric acid- produced by *Rhizopus* spp. in almond hull rot disease
- Oxalic acid -*Sclerotium* and *Sclerotinia* spp. in various plants they infect and by *Cryphonectria parasitica*, the cause of chestnut blight
- Alternaric acid, alternariol, and zinniol -*Alternaria* spp. in leaf spot diseases of various plants
- Ceratoulmin- *Ophiostoma ulmi* in Dutch elm disease

- Fusicoccin- *Fusicoccum amygdali* in the twig blight disease of almond and peach trees
- Ophiobolin -several *Cochliobolus* spp. In diseases of grain crops
- Pyricularin- *Pyricularia grisea* in rice blast disease
- Fusaric acid and lycomarasmin -*Fusarium oxysporum* in tomato wilt

Host-specific or host-selective toxins

- A **host-specific** or **host-selective** toxin is a substance produced by a pathogenic microorganism that, at physiological concentrations, is toxic only to the hosts of that pathogen and shows little or no toxicity against non-susceptible plants.
- Most host-specific toxins must be present for the producing microorganism to be able to cause disease.
- Host-specific toxins have been shown to be produced only by certain fungi (*Cochliobolus*, *Alternaria*, *Periconia*, *Phyllosticta*, *Corynespora*, and *Hypoxylon*), although certain bacterial polysaccharides from *Pseudomonas* and *Xanthomonas* have been reported to be host specific.

Victorin, or HV Toxin

- Victorin, or HV-toxin is produced by the fungus *Cochliobolus (Helminthosporium) victoriae,* which causes Victoria blight of oats.
- This fungus appeared in 1945 after the introduction and widespread use of the oat variety Victoria and its derivatives, all of which contained the Vb gene for resistance to crown rust disease.
- *C. victoriae* infects the basal portions of susceptible oat plants and produces a toxin that is carried to the leaves, causes a leaf blight, and destroys the entire plant.
- Victorin is a complex chlorinated, partially cyclic pentapeptide.
- The primary target of the toxin seems to be the cell plasma membrane, where victorin seems to bind to several proteins.
- Victorin also functions as an elicitor that induces components of a resistance response that include many of the features of hypersensitive response and lead to programmed cell death.

T Toxin [HMT Toxin]

- T toxin is produced by race T of *Cochliobolus heterostrophus* (anamorph: *Bipolaris maydis,* earlier called *Helminthosporium maydis*), the cause of southern corn leaf blight.
- First appeared in the United States in 1968, it spread throughout the corn belt by 1970, attacking only corn that had the Texas male-sterile (Tms) cytoplasm.

- The ability of *C. heterostrophus* race T to produce T toxin and its virulence to corn with Tms cytoplasm are controlled by one and the same gene.
- T toxin does not seem to be necessary for the pathogenicity of *C. heterostrophus* race T, but it increases the virulence of the pathogen.
- The T toxin apparently acts specifically on mitochondria of susceptible cells, which are rendered nonfunctional, and inhibits ATP synthesis.

HC Toxin

- Race 1 of *Cochliobolus carbonum* (anamorph:*Bipolaris(Helminthosporium)* *zeicola*) causes northern leaf spot and ear rot disease in maize.
- It also produces the host-specific HC toxin, which is toxic only on specific maize lines.
- The mechanism of action of HC toxin is not known, but this is the only toxin, so far, for which the biochemical and molecular genetic basis of resistance against the toxin is understood.
- Resistant corn lines have a gene (Hm1) coding for an enzyme called HC toxin reductase that reduces and thereby detoxifies the toxin.
- Susceptible corn lines lack this gene and, therefore, cannot defend themselves against the toxin.

***Alternaria alternata* Toxins**

- Several pathotypes of *Alternaria alternata* attack different host plants and on each they produce one of several multiple forms of related compounds that are toxic only on the particular host plant of each pathotype.
- Some of the toxins and the hosts on which they are produced and affect
    - AK toxin causing black spot on Japanese peat fruit,
    - AAL toxin causing stem canker on tomato,
    - AF toxin on strawberry,
    - AM toxin on apple,
    - ACT toxin on tangerine,
    - ACL toxin on rough lemon, the HS toxin on sugar cane.
    - As an example of *A. alternata* toxins, the AM toxin is produced by the apple pathotype of *A. alternata*, known previously as *A. mali*, the cause of Alternaria leaf blotch of apple.

Lecture 17

HOST PARASITE INTERACTION

Aim: To acquaint the students with host parasite-interaction

Host Pathogen Interactions

- After the pathogen enters the host until the disease symptoms appear, a series of interactions between host and pathogen take place.
- The disease symptoms may be considered as the signs of reaction of the host to infection by the pathogen.
- The severity of the symptoms varies depending upon the capacities of each of them to supersede the other.
- In that process, which may last for a few hours to many days, different biochemical reactions become involved.
- The establishment of the pathogen or colonization of the host by the pathogen after its entry initiates the symptoms.
- The **hypersensitive reaction** of the host is a mechanism to prevent such colonization by the pathogen.
- Many pathogens which cause disease in plants are highly specific in their nutritional requirements.
- They must reach the proper site inside the host tissue to obtain the required nutrients.
- The chances of a pathogen establishing itself in a host depend on:
    - its entering the suitable host
    - its reaching the proper location within the host tissue so that the required nutrients are obtainable
- A vascular pathogen may not establish itself inside a host if it is confined to sub-stomatal region.
- Similarly, wood rotting basidiomycetous fungus may not find food for its growth if it enters the leaf tissues.
- Tissue preference of parasites has been, for a long time, attributed to specific nutrients present in that particular tissue.
- Recent evidence indicates that tissue preference is solely influenced by inhibitory substances **(prohibitins)** present in the tissue.

Post Entry Stages

- The subsequent stages, after the pathogen has entered a susceptible host which is pre-disposed to the disease, are of two kinds:
    - The pathogen may kill the host tissue in advance, drawing nutrients from the dead cells.
    - The pathogen and host may develop an harmonious relationship or association in which neither is killed. The pathogen absorbs nutrients from the living host cells.
- Another biological relationship which exists is nature in the case of root nodule bacteria and legume plants is **symbiosis**, whereby two biological systems derive benefit from each other.

Effects of pathogens on photosynthesis

- The pathogen directly affects the photosynthetic capacity of the host.
- In many cases the pathogen causes chlorosis of the tissues, indicating through inhibition of certain enzyme activities.
- The Hill reaction, in which water is split into oxygen and hydrogen atoms, which in turn, is coupled with the production of adenosine triphosphate (ATP) through photosynthetic phosphorylation is adversely affected.
- Apparently the enzymes of CO_2 fixation in photosynthesis are not affected, but the glycolic acid oxidases are affected, resulting in a reduction in protein synthesis.
- Because of the reduction in photosynthetic activity, other chain reactions in the plant result, causing increased water loss, wilting and reduction of plant vigour.

Effects of pathogens on respiration

- In general, plants infected by most pathogens react with an immediate increase in the respiratory rate.
- This increase is mostly non-specific, as even mechanical injuries to certain tissues also cause it.
- In incompatible and destructive host-pathogen relationships, the respiratory rate is high in the early stages of infection, whereas in compatible host-pathogen relationship, as in obligate parasitism, there is little change in the respiratory rate.
- In the destructive host-pathogen relationship, the pathogen destroys the normal physiological balance, the **Pasteur effect**, (which implies that in the presence of oxygen the fermentative degradation of carbohydrates is reduced and the energy release is more) is abolished, and hence, the respiratory efficiency is also reduced.

Effect of pathogens on Embden-Meyerhof-Parnas pathway

- There is an accumulation of carbohydrates, mostly starch, in the infected tissues, coupled with a shift from the Embden-Meyerhof-Parnas pathway to the pentose-phosphate cycle which causes the accumulation of reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$).
- The pathogen causes tissue disintegration of the host, which is accomplished by the activity of several oxidative enzymes such as peroxidase, phenol oxidase and ascorbic acid oxidase, linked up with the oxidation of $NADPH_2$ to NADP.
- The hypersensitive reaction in a plant that results in necrosis also causes a shift in the respiratory pathway from the Embden- Meyerhof-Parnas to the pentose phosphate system.
- There is also an increase in the oxidative enzyme activity to cause an enhancement of oxygen uptake. If certain oxidative enzyme inhibitors are produced in the host-pathogen system, the resistance of the host plant is reduced, and in the absence of oxidative enzyme inhibitors, the resistance of the host is increased.

Pathogenesis related proteins

- Infection by pathogens also interferes with the host nucleic acid and protein metabolism, especially enzymes.
- Most conspicuous changes are in peroxidase, ascorbic acid oxidase, cytochrome oxidase, phenol oxidase, etc.
- In addition, several proteins accumulate which were first called **pathogenesis related proteins** and are now referred to as **stress proteins** as they accumulate in response to physical and biological stresses.
- Both in virus infected tissues and galls, nucleic acids especially RNA accumulate.
- Hypertrophy of host cells, accompanied by increases in the size of nuclei has been demonstrated.
- There is redirection of protein synthesis towards accumulation of proteins of the pathogen, or a reduction in the protein and nitrogen level of the host, especially towards the degenerative stages of disease.
- The synthesis of virus protein markedly affects the host protein metabolism. In hypersensitive reaction, however there is no great change in the total protein content or nitrogen level in the affected tissues.

Phytoalexins

- The phenol metabolism of infected plants is profoundly altered.

- In incompatible host-pathogen combinations, there is more rapid accumulation of phenolic substances than in compatible combination.
- Certain phenolics and other aromatic substances with antimicrobial properties, named **phytoalexins**, are produced post-infectionally in host tissues, and this is believed to be a response to infection directed towards imparting resistance in the host.
- This response is greater in incompatible host-pathogen combination than in the compatible one.
- Production of phytoalexins may also be induced in the host in non-pathological conditions, such as physical stress and chemical stimuli.
- The production of leaf spots, necrosis and other types of lesions on the host are correlated with enhanced phenol oxidase activity. Oxidation of phenolic substances causes the accumulation of melanin pigments in the infection site, resulting in discoloration.

Growth regulators

- Due to pathological condition of the plant, the growth regulatory mechanism is upset and depends largely on the nature of host –plant relationship.
- **Auxin** level often increases in the host tissue as a result of infection by fungi and bacteria.
- This may be due to increased auxin synthesis by the host and /or pathogen, or the suppression of activity of enzymes indole acetic acid oxidase which degrades the auxins under normal conditions.
- **Gibberellins** or gibberellin-like substances have been found in many plants and are also produced by a few fungi.
- The auxin activity in meristematic tissues of plants is believed to be controlled by gibberellins.
- Infection of plants with some pathogens alters the level of ***gibberellin-like substances*** which result in the hyper-elongation of the plant.
- One such typical example is the Bakane disease of rice plants caused by *Fusarium moniliforme* (teleomorph: *Gibberella fujikuroi*).
- Dwarfism or stunting in some host-pathogen interactions is caused by a reduction in the gibberellins.
- **Kinetin (cytokinin)**, 6-furfuryl aminopurine, an essential hormone, is involved in cell division in plant tissues.
- IAA and cytokinin act in a linked manner, the former responsible for cell expansion and the latter for cell division.

- In the absence of IAA, however, kinetin seems to be functionless.
- From plants and microorganisms, cytokinin has been isolated and characterized.
- It is a precursor of tRNA. Certain plant pathogens interfere with normal cytokinin metabolism in plants causing gall formation, senescence, fasciations, yellowing of leaves, green island formation, etc. which could be reversed by the exogenous application of kinetin.
- **Ethylene,** another well known growth regulator in plants plays a prominent role in causing epinasty, leaf yellowing and senescence in diseased plants.
- Many plant pathogens favour accumulation of high levels of ethylene in host tissues which contribute to symptom development like leaf drop, e.g., due to *Diplocarpon rosae*, the black spot of rose pathogen.

Lecture 18

VARIABILITY IN PLANT PATHOGENS

Aim: To acquaint the students with the concept of variability in plant pathogens

Variability means the quality of being subject to variation or quality of being uneven or lacking uniformity. In plant pathogens, the variation takes place due to genetic variability. Genetic variability is the measure of the tendency of individual genotype in a population to vary from other or having more than one genetic state or allele at each locus.

- Variation is usually associated with sexual reproduction by fungal oospores, zygospores,
- a **variant** can be defined as an individual among a certain population that have deviated from the normal individuals genetically and possesses certain different characters which are not generally associated with that particular population previously.

Mechanisms of variability in plant pathogens

- General mechanisms of variability are:
  -mutation, recombination, gene and genotype flow, genetic drift and selection, out-crossing etc.
- Specialized mechanisms of variabilityare:
  -heterokaryosis, parasexuality, saltation etc. in fungi
  -conjugation, transformation and transduction in bacteria
  - recombination in viruses

1. General mechanisms of variability in fungi

Mutation is more or less an abrupt change in genetic material of an organism or a virus, which is then transmitted in a hereditary fashion to the progeny.

- Mutations in nature are less frequent and are result of infrequent changes that take place during cell division and result in irregularities in replication or rearrangement of minute parts of genetic material of the cells.
- Mutation can be induced artificially with increased frequency by physical agents like ultra violet rays, X-rays, gamma-rays or by chemicals like alkaloids, phenols etc.
- Most mutations are recessive; therefore in diploid organisms mutation remains unexpressed until they are brought together in a homozygous condition.

- Although frequency of mutation is low, but given the great number of progeny produced by pathogen, it is possible that large number of mutants differ in virulence from their parents.
- Mutation has been reported in *Cladosporium fulvum* causing tomato leaf mould, *Phytophthora infestans*, *Puccinia graminis* and *Melampsora lini*, apart from the appearance of highly destructive race T of *Heliminthosporium maydis.*

Recombination occurs primarily during the sexual reproduction of plants, fungi, and nematodes whenever two haploid nuclei containing genetic material that may differ in many loci unite to form a diploid nucleus called a zygote.

- Recombination can be intraspecific, interspecific or even intergeneric; and the resulting hybrids may have different pathogenic abilities from the parental races.
- Often the hybrids are intermediate in pathogenicity between the two parental races, but some may be more pathogenic than others; and similar considerations apply to other inherited characteristics.
- Recombination of genes occurs in autoecious rusts, such as *Melampsora lini.* Similarly, evolution of new physiological races through meiotic recombination is common in many pathogenic fungi, rusts, smuts, powdery mildews apart from potato blight fungus.

Gene flow is a process by which certain alleles move from one population to another geographically separate population.

- **Population genetics, genetic drift and selection** also bring about variability in the plant pathogens.
- **Mating system** is considered in terms of the amount of inbreeding that occurs in a population of sexual organism. Many smut fungi are forced to inbreed because a dikaryon must form for a successful infection and likely encounters are between the basidiospores arising from same psuedobasidia of the strains in the soil.
- Outcrossing of individuals put together new combination of genes rapidly leading to many different genotype within population.

2. Specialized mechanism of variability in fungal pathogens

Heterokaryosis is occurrence of dissimilar or genetically different nuclei in a vegetative cell or spore or hypha as in basidiomycetes.

- It provides haploid organisms the ability or somatic flexibility with changing environment.
- It increases diversity as genetic recombination is brought about by interchanges of whole chromosome or through mitotic crossing-over.
- It plays an important role in homothallic and imperfect fungi.
- Heterokaryosis can arise by

  a) gene mutation

  b) fusion of vegetative mycelium

  c) at the time of spore formation in many fungi such as *Neurospora tetrasperma*, *Podospora* sp.
- Heterokaryosis is certainly a way in which avirulent strains may acquire virulence, for example in *Thanatephorus cucumeris.*

Parasexuality is a process in which genetic recombination occurs in the vegetative thallus in absence of sexual stage.

- Sequence of events in parasexuality is as follows:

  i) formation of dikaryotic mycelium

  ii) fusion between two nuclei

  iii) multiplication of diploid nuclei side by side the haploid nuclei

  iv) occasional mitotic crossing over during the multiplication of diploid nuclei

  v) sorting out of diploid nuclei

  vi) occasional haploidization of the diploid nuclei

  vii) sorting out of the new haploid strain.
- Parasexuality has produced new races of *Fusarium oxysporum* f. sp. *pisi*, *Ascochyta*, *Verticillium albo-atrum* etc.

Saltation is appearance of morphologically different sectors in fungal colonies.

- It occurs frequently in fungal colonies of some isolates of *Fusarium* and *Helminthosporium.*
- Saltation may be influenced by compaction and thickness of culture media.

3. Sexual-like processes in bacteria

New biotypes of bacteria seem to arise with varying frequency by means of at least three sexual-like processes.

i) In **conjugation,** two compatible bacteria come in contact with each another and a small portion of the chromosome or plasmid from one bacterium is transferred to the other through a conjugation bridge or pilus.

ii) In **transformation**, bacterial cells are transformed genetically by absorbing and incorporating in their own cells genetic material secreted by or released during rupture of other bacteria.

iii) In **transduction**, a bacterial virus (phage) transfers genetic material from the bacterium in which the phage was produced to the bacterium it infects next.

- When the gene transfer is limited to members of the same species or even genus, it is called **vertical gene transfer**. Sometimes, gram-negative bacteria can transmit genetic material readily across species; as *Agrobacterium* transmits genes across kingdom barriers to plants, such events are called **horizontal gene transfer**.

4. Genetic recombination in viruses

- When two strains of the same virus are inoculated into the same host plant, one or more new virus strains are recovered with properties (virulence, symptomatology, and so on) different from those of either of the original strains introduced into the host.

- The new strains probably are recombinants, although their appearance through mutation, not hybridization, can not always be ruled out.

- In multipartite viruses consisting of two, three, or more nucleic acid components, new virus strains may also arise in host plants or vectors from recombination of the appropriate components of two or more strains of such viruses.

5. Loss of pathogen virulence in culture

- The virulence of pathogenic microorganisms toward one or all of their hosts often decreases when the pathogens are kept in culture for relatively long periods of time or when they are passed one or more times through different hosts.
- If the culturing of the pathogen is prolonged sufficiently, the pathogen may lose virulence completely. Such partial or complete loss of virulence in pathogens is sometimes called **attenuation**, and it has been shown to occur in bacteria, fungi and viruses.
- Loss of virulence in culture, or in other hosts, seems to be the result of selection of individuals of less virulent or avirulent pathogen strains that happen to be capable of growing and multiplying in culture, or in the other host, much more rapidly than virulent ones.
- After several transfers in culture or the other hosts, such attenuated individuals largely, or totally, overtake and replace the virulent ones in the total population so that the pathogen is less virulent or totally avirulent.
- On reinoculation of the proper host, isolates in which the virulent individuals have been totally replaced by avirulent ones continue to be avirulent, and therefore loss of pathogenicity is irreversible.
- However, on reinoculation of the proper host with isolates in which at least some virulent individuals survived through the transfers in culture or the other host, the few surviving virulent individuals infect the host and multiply, often in proportion to their virulence.

- The virulent individuals increase in number with each subsequent inoculation; while at the same time, non-virulent individuals are reduced or eliminated with each reinoculation.

Stages of variation in pathogens

- **Species:** The entire population of a particular organism on the earth, e.g. a fungal pathogen, has certain morphological and other phenotypic characteristics in common and makes up the species of pathogen, such as *Puccinia graminis*, the cause of stem rust of cereals.
- **Varieties or special forms:** Some individuals of this species, however, attack only wheat, barley, or oats, and these individuals make up groups that are called varieties or special forms (*forma specialis*) such as *P. graminis* f. sp. *tritici* or *P. graminis tritici*, *P. graminis hordei*, and *P. graminis avenae*.
- **Race:** Even within each variety or special form, some individuals attack some of the varieties of the host plant but not the others, some attack another set of host plant varieties, and so on with each group of such individuals making up a race. Thus, there are more than 200 races of *P. graminis tritici* (race 1, race 15, race 59 and so on).
- **Variant:** Occasionally, one of the off-spring of a race can suddenly attack a new variety or can cause severe symptoms on a variety that it could barely infect before. This individual is called a variant.
- **Biotype:** The identical individuals produced asexually by the variant make up a biotype. Each race consists of one or several biotypes (race 15A, 15B and so on).

Appearance of new pathogen biotypes

- The appearance of new pathogen biotypes may be very dramatic when the change involves the host range of the pathogen.
- If the variant has lost the ability to infect a plant variety that is widely cultivated, this pathogen simply loses its ability to procure a livelihood for itself and will die without even making its existence known to us.
- If, however, the change in the variant pathogen enables it to infect a plant variety cultivated because of its resistance to the parental race or strain, the variant individual, being the only one that can survive on this plant variety, grows and multiplies on the new variety without any competition and soon produces large populations that spread and destroy the resistance variety.
- This is the way the resistance of plant variety is said to be broken down although it was the change in the pathogen, not the host plant that brought it about.

Lecture 19

DISEASE RESISTANCE AND DEFENCE MECHANISMS IN PLANTS

Aim: To acquaint the students with the concepts of disease resistance and defence mechanisms in plants

Disease resistance

- Resistance is the ability of a host plant to resist the growth or establishment of a pathogen.
- Many plant pathogenic microorganisms are specific to certain host plants.
- The plant species and cultivars vary widely in their capacity to resist the establishment of a pathogen.
- When a pathogen enters the host, it must overcome several barriers before establishing itself.
- Sometimes the host reacts to the entrance of the pathogen or alters growth of the pathogen and thus effectively checks the establishment of the disease.
- There is great variation in the degree of resistance of a plant to a given parasite.
- This is influenced by various factors, involving the host, parasite and environment.

Disease escape: When the plant, though genetically susceptible to the pathogen, may show resistance in the field; such a condition is termed as **disease escape**.

- It happens when the optimum conditions are not present for the disease development on the crop variety, or the crop may not be in the proper stage of growth when the pathogen is most active.
- The host plant may be susceptible to the pathogen when it is young and develop resistance at maturity or vice-versa.

Disease endurance: In some cases, the plant is capable of carrying on most of the normal metabolic processes in spite of the diseased condition without reducing the yield to a considerable extent. It is known as **disease endurance or disease tolerance**.

Hypersensitive reaction or hypersensitivity: In hypersensitivity, when the pathogen enters the host, the cells in the immediate vicinity react in such a manner as to delimit the spread of the pathogen through death reaction of the cell, or by forming other barriers.

- The symptoms of such reactions in the host appear as minute specks, which the indicative of high resistance in the plant.
- The plant may be called resistant to the disease, if it shows **hypersensitive reaction** to infection by the pathogen.

Types of resistance

Resistance to diseases is also a genetically controlled character. Plants possess two different types of resistance:

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

Vertical vs. horizontal resistance

- The concept of **vertical** and **horizontal resistance** was suggested by Vander Plank in 1968.
- Resistance is vertical (differential) when it is completely effective against some races of a pathogen but not against the others.
- Vertical resistance is complete but is not permanent.
- Horizontal resistance is effective against all races of a pathogen.
- Horizontal resistance, though incomplete, is of permanent nature.

Conceptual explanation of different terminologies used to explain vertical and horizontal resistance by research workers are illustrated hereunder:

Science	**Type of Resistance**	
Genetics	Monogenic	Polygenic
Plant Breeding	Non-durable	Durable
Agronomy	Seedling	Adult plant
Epidemiology	Vertical	Horizontal

Gene for gene hypothesis

- It was first proposed by Flor (1942, 1945) as the simplest explanation of the result of studies of the inheritance of resistance and pathogenicity flax-rust host-pathogen system, and now applies to most of the combinations.

- According to this hypothesis, **"for a resistance gene in the host there is a complementary avirulence gene in the pathogen."**

- The coexistence of host plant and pathogen side by side in nature indicate that they have co-evolved; and changes in virulence of pathogen have been continuously governed by the changes in host or vice versa.

- This concept has been shown to operate in many other diseases like rusts, smuts, apple scab, late blight of potato and many other fungal , bacterial , virus and higher parasitic plant diseases.

- Generally, but not always in the host, gene for resistance is **dominant (R)** and gene for susceptibility is **recessive (r).**
- In pathogen, however, gene for avirulence that have inability to infect is **dominant (A)** and for virulence it is **recessive (a).**
- Thus plant variety when carrying gene for resistance (R) for certain pathogen and other lacking gene (R) i.e. carrying gene for susceptibility (r) are inoculated with two races of pathogen, one of which carries a gene for avirulence (A) and other carrying the gene for virulence (a) against (R); it gives a 4 gene combinations as below:

	R	r
A	AR (-)	Ar (+)
a	aR (+)	ar (+)

(-) denotes incompatible (resistant reaction); (+) denotes (compatible) susceptible reaction

- Out of the four combinations as above; only **AR** interaction is resistant or incompatible i.e. host has certain gene for resistance(R) that recognizes the corresponding genes for avirulence (A), so there is incompatibility.
- In **Ar** combination, infection results because host lacks the gene for resistance so pathogen can attack with other gene for virulence.
- In **aR** combination, infection occurs, although the host has gene for resistance but the pathogen lacks the gene for avirulence (A) which is recognized by specific gene for resistance; hence no defence mechanism is activated.
- Finally in **ar** gene combination, as plant lacks the gene for resistance and have gene for susceptibility (r) and pathogen have the gene for virulence (a), it results in infection.

Genetics of resistance

- Gene for resistance appear and accumulate first in host through evolution and they coexist with non-specific genes for pathogenicity that exist in pathogen.
- Genes for pathogenicity exist in pathogen against all host plants that lack specific resistance.
- When a specific gene for resistance appears in the host or bred into the host, the gene enables the host to recognize the product of the particular gene for virulence in pathogens, the pathogen gene is then thought of as the virulence gene (avrA) of pathogen that correspond to plant resistance gene (R).
- The change in the function of the pathogenic gene is because of subsequent recognition of avrA product i.e. elicitor molecule by the receptor coded by R gene which triggers the hypersensitive response reaction in the plant that keeps the plant resistant.

Breakdown of resistance

- A new gene for virulence that attacks existing gene for resistance appears by mutation of an existing avr gene which then avoids gene for gene recognition and the resistance breaks down.
- Plant breeders introduce another gene for resistance (R) in plant which recognizes the protein of new gene for virulence of pathogen and extend the resistance of host beyond the range of new gene for virulence in pathogen.
- This produces a variety that is resistant to all races that have an avirulence gene corresponding to specific gene for resistance until another gene for virulence appears in pathogen.

Defence mechanisms in plants

- Resistance to infection has been attributed to morphological and structural feature of plants.
- Evidences, however, are very much against their participation in resistance.
- The essential character of resistance is a result of physiological activity of plants and the resistance is displayed through substance called prohibitins.
- **Prohibitins -** inhibit and also inactivate extra-cellular enzymes and toxin of parasites, and prevent their spread.
- Most of the prohibitins are phenolic in nature; they react with enzymes and inactivate toxins by forming complexes.
- Examples are: allicin produced in garlic, catechol and protocatechuic acid in onions, chlorogenic acid and caffeic acid in coffee, mangiferin in mango, and phloretin in apple.
- **Phytoalexins**
- During infection, most plants synthesize toxic substances which form the basis of phytoalexin theory.
- **Phytoalexins** are "low molecular weight substances produced by plants relatively rapidly and in high concentration in response to infection by pathogens, and physico-chemical stress which are active in a wide range of pH, temperature and nutrient conditions; and confer protection against the infecting parasites.
- Ipomeamarone produced in sweet potato, rishitin in tomato, phaseollin in beans and pisatin in peas are some examples of such compounds.
- They are confined to the tissue colonized by the pathogen.
- The speed with which phytoalexins are produced depends upon the degree of resistance in the host.
- The sensitivity or capacity of the host to produce phytoalexins is governed by genetic factors; and a gene-for gene relationship in host parasite reactions appears to be present.

Structural defence

- A few host plants produce **structures** in response to infection which prevent the spread of the pathogen.
- These structures are **cork layers**, **tyloses** and **abscission layers**.
- Deposition of gums, resins, polymers of tannins and melanin, swelling of cell walls and their sheathing in columns are other defense reactions.
- Cork layers are produced by potato tubers infected by *Rhizoctonia solani*, which prevent the spread of toxic substances, produced by the pathogen and effectively check the flow of nutrients and water from the healthy to the infected area.
- Tyloses formation has been noted in tomato and sweet potato plants against wilt pathogens.
- Formation of abscission layer is a wide spread response of plants infected by fungi, bacteria or viruses.
- Gum deposits impregnated with toxic substances along the borders of lesions frequently check the advance of parasites; a typical example being the resistance of rice to blast pathogen *Pyricularia oryzae*.
- Sheathing of infecting hyphae by extension of the cell walls has been observed in a few instances. The sheath consists of cellulose, callose substances and lignin.

Lecture 20

DISSEMINATION OF PLANT PATHOGENS

Aim: To acquaint the students with the dissemination or dispersal of plant pathogens

Dissemination is the spread of plant pathogens within the general area in which it is established is termed as their dissemination or dispersal or transmission.

Methods of dissemination

- Different methods of dissemination of the plant pathogens with in a crop season as well as to the next season after its survival are:

i) Direct methods

ii) Indirect methods.

- In direct transmission, the dispersal takes place along with the seeds and vegetative plant parts used for propagation.
- Indirect transmission may be active/autonomous or brought about passively by different agencies like wind, water, animals or human beings.

Direct transmission

It is further divided into:

- Adherent transmission
- Germinative transmission
- Vegetative transmission

- In **adherent** type, the pathogen propagules are carried over the surfaces of seed or other propagative materials. Bunt of wheat caused by *Tilletia foetida* and *T. caries* is carried through the seed externally. Similarly, spores of *Synchytrium endobioticum* which causes potato wart and *Rhizoctonia solani* (the black scurf pathogen) sclerotia are adherent to potato tubers used as seeds.
- In **germinative** type, the plant pathogens are carried through the seed or other propagules internally as in case of loose smut of wheat and barley. Similarly, bean mosaic is transmitted through pollen grains and is carried in the seeds.

- In **vegetative** transmission, a large number of fungal, bacterial, viral and phytoplasmal plant pathogens are carried in the vegetative plant parts used as seeds such as tubers, cuttings, runners, grafts etc.
- For example, ring rot of potato caused by *Clavibacter sepidonicum* and *Ralstonia solanacearum* causing brown rot is carried through infected potato tubers to new ones.
- Similarly, dormant mycelium of *Phytophthora infestans* is carried through potato tubers. A large number of viruses, e.g., potato virus X, potato virus Y, citrus tristeza and apple mosaic virus, etc. are transmitted through their propagative parts apart from red rot and whip tail of sugarcane.

Indirect transmission

It can be:

a. Autonomous/Active transmission

b. Wind dispersal of pathogens (Anemochory)

c. Water dispersal of pathogens (Hydrochory)

d. Animal dispersal (Zoochory)

e. Insect dispersal (Entomochory)

f. Human dispersal (Anthropochory)

Autonomous transmission

- By this method, the plant pathogens are spread to short distances.
- It takes place by active growth of the hyphae or hyphal strands.
- It is characteristic of wood rotting fungi like *Arnillariella*, *Fomes*, *Ganoderma*, etc. which migrate independently through the soil from plant to plant or even from field to field by active growth of their strands.
- Zoospores of *Phytophthora* and *Pythium* can swim through the water film in soil although to limited distances, likewise nematodes.
- Spores of some fungi are expelled forcibly from the sporophores or sporocarps by puffing action.
- The seeds of some parasitic plants are also expelled forcibly and may reach over distances of several meters.

Dissemination by wind/air

- Many fungal spores and seeds of most parasitic plants are disseminated by wind or air currents that carry them as particles to various distances.
- Air current pickup spores and seeds of sporophores and carry them upward or horizontally.
- While air borne some of the spores may reach the wet surfaces and get trapped; and when air movement stops or it rains the rest of the spores land or may be brought down by rain drops on to the susceptible host surfaces.
- The spores of many fungi are too delicate to survive a long trip through air and are, therefore, successfully disseminated to a few hundred to a few thousand meters only.
- The spores of other fungi, particularly those of cereal rusts are very hardy and can be successfully transported over distances of several kilometers for causing widespread epidemics.
- Bacteria and nematodes present in the soil may be blown away along with soil particles on the dust storm.
- Wind also helps in the dissemination of bacteria, fungal spores and nematodes by blowing away rain splash droplets carrying them and wind also carries away insects that may contain or smeared with viruses, bacteria or fungal spores.

Disseminations by water

- Water is an important agency for disseminating pathogens in three ways:
- Bacteria, nematodes and spores, sclerotia and mycelial fragments of fungi present in soil are disseminated by rain or irrigation water that moves on the surface or through the soil.
- All bacteria and spores of many fungi are exuded in a sticky liquid and depend for the dissemination on rain or over-head irrigation water which either washes them downward or splashes them in all directions.
- Rain drops or drops from over-head irrigation pickup the fungal spores and any bacterial propagules from the air and wash them downward where some of them may land on susceptible plants.

Dissemination by insects

- Insects, particularly aphids and leaf hoppers are by far the most important vectors of viruses, phytoplasmas and fastidious bacteria.

- Each one of these pathogens is transmitted internally by only one or a few species by insects during feeding and movements of insect vectors from plant to plant.
- More than 50 species of aphids are known to transmit the viruses of plants. Some can transmit only a few kinds or other transmits many.
- *Myzus persicae*, the green peach aphid alone is the vector of fifty different viruses. Some of the diseases transmitted by aphids are Katte or Marble disease of cardamom, bunchy top of banana, cowpea mosaic, papaya mosaic, potato leaf roll.
- Most of the yellows and witches' broom type of diseases are transmitted by leaf hoppers including rice tungro.
- Other insects like white flies transmit some important diseases like leaf curl of cotton, tobacco etc. Bottle gourd mosaic is transmitted by red pumpkin beetle.
- Thrips transmit the spotted wilt virus; whereas squash mosaic, cowpea mosaic and turnip yellow mosaic viruses are transmitted by beetles while the turnip yellow mosaic virus (TYMV) is also transmitted by grass hoppers and ear wigs.
- In general, the viruses that require no incubation period in the insect vectors are called **non persistent or stylet borne viruses**; and those which enter into the system of the insects and require incubation period are called **persistent or circulative viruses.**
- Insects are also important in the dissemination of certain bacterial and fungal plant pathogens. *Erwinia tracheiphila,* the cucurbit wilt organism in completely dependent on cucumber beetle for its spread. *Xanthomonas stewartii,* the corn wilt pathogen overwinters inside the corn flea beetle. Insects are also important agent of dissemination and inoculation of *Erwinia carotovora* causing black leg of potato. *Erwinia amylovora* causing fire blight of pear and apple is known to be transmitted by ants, whereas *Xanthomonas citri* causing citrus canker is carried by leaf mite from diseased to healthy plants. The conidia of *Claviceps* are spread from malformed to healthy flowers by insects feeding on honey dew. The spores of *Ceratocystis fagacearum* (oak wilt) and *Ceratocystis ulmi* (Dutch elm disease) are carried by beetles.

Dissemination by Nematodes

- About 20 viruses are known to be transmitted by the members of 4 genera of soil borne ectoparasitic nematodes, viz., *Longidorus, Xiphinema, Trichodorus* and *Paratrichodorus. Longidorus* and *Xiphinema* spp. transmit polyhedral-shaped viruses (NEPO) such as tobacco and tomato ring spots, cherry roll, grape vine fan leaf and others. *Xiphinema index* transmits fan leaf disease of grapevine. Whereas, *Trichodorus* and *Paratrichodorus* spp. transmit tubular (NETU) viruses like and tobacco rattle and pea early browning.

- Some fungal and bacterial plant pathogens are also transmitted by nematodes, e.g., *Corynebacterium fasciens* causing leaf gall disease is transmitted by ectoparasitic nematodes *Aphelenchoides*. *Anguinia tritici* is believed to transmit fungal pathogen *Dilophospora alopecuri* causing leaf spotting and twist of grains and cereals.

Dissemination by mites

- Mites transmit both stylet-borne and circulative viruses, e.g., wheat streak mosaic virus, peach mosaic virus, sterility disease of pigeon pea.

Dissemination by animals

- Almost all animals small and large that move among plants and touch them along the way can disseminate pathogens such as fungal spores, bacteria, seeds of parasitic plants, nematodes and some viruses and viroids.
- Most of these pathogens adhere to the feet or the body of the animals, but some may be carried in contaminated mouth parts.

Dissemination by fungi and dodder

- Some plant pathogens, like the zoospores of some fungi (e.g. *Olpidium brassicae*) and certain parasitic plants (e.g. dodder) can transmit viruses as they move from one plant to the others (zoospores) or they grow and form a bridge between two plants.
- *Olpidium brassicae* can transmit lettuce big vein virus, tobacco necrosis and tobacco stunt viruses.
- *Synchytrium endobioticum* can transmit PVX and potato mottle virus whereas dodder can transmit many viruses including aster yellows.

Dissemination by human beings

- Humans are known to disseminate all kinds of pathogens over short and long distances in a variety of ways.
- Humans disseminate some pathogens such as tobacco mosaic virus by successively handling of diseased and healthy plants.
- Other pathogens are disseminated through farm tools such as pruning shears (pear fire blight), ploughs etc. by contaminating the healthy plants or plant parts after their contamination with spores and other pathogen structures.
- Humans also transport pathogens by contaminated soil on their feet or equipment and by using infected transplants, seed, nursery stock and bud wood.
- Humans disseminate plant pathogens by importing new varieties in to an area that may carry pathogens that have gone undetected by travelling throughout the world and by importing food or other items which may carry harmful plant pathogens.
- For examples, introduction of Dutch elm disease (caused by *Ophiostoma ulmi*) or citrus canker in USA or powdery mildew of grapes in Europe; and more recently rapid spread of ergot of sorghum through out the world.
- Similarly, deadly diseases like late blight of potato (1883), downy mildew of grapes (1910) and bacterial blight of rice (1959) were introduced in England, Europe and Philippines, respectively by human activity only.

Lecture 21

SURVIVAL OF PLANT PATHOGENS

Aim: To acquaint the students with survival of plant pathogens

Survival

To continue the infection chain, most of the plant pathogens have developed some efficient means of survival through the unfavourable part of the year. So that with the onset of the favourable season its infection may be renewed. Chief sources of survival of plant pathogens are:

- Infected living hosts
- Infected or contaminated planting organs
- Infected crop residues
- Resting structures
- Soil

Infected living hosts

- Infected plants are the most important sources/reservoir of inoculum for plant diseases.
- Bacteria, fungal spores and spore producing structures such as pycnidia, acervuli etc. may survive the unfavorable season in the infected twigs and branches of perennial plants, e.g., *Erwinia amylovora* in apple, *Xanthomonas campestris* pv. *citri* in citrus causing canker.
- When the favourable season returns, the resting bodies come out of their dormant state, become active and produce primary inoculum.
- Some crops like rice, chillies, brinjal or brassicas are grown throughout the year and provide continuity to the pathogens infecting them year after year.
- Also, the volunteer or self sown plants growing outside the fields get infected during the off-season and may act as a good source of inoculum to the new crop, e.g., *Puccinia graminis* on wheat, *Helminthosporium oryzae* and *Pyricularia oryzae* on rice or *Alternaria solani* on chillies.

Subsidiary hosts

- Many plant pathogens have a wide host range and attack different related or unrelated plant species grown in different situations and different season. These are called subsidiary hosts. These include:

  a) Collateral hosts

  b) Alternate hosts

  c) Wild hosts of the same family

  d) Weed hosts.

Collateral hosts

- Fungal pathogens like *Alternaria solani* and *A. brassicicola* mostly attack members of *Solanaceae* and *Brassicae* family, respectively, which are their collateral hosts.

Alternate hosts

- *Puccinia graminis tritici* which causes stem rust of wheat attacks and survives on barberry (*Barberis vulgaris)*, the only other species it affects other than wheat.
- Such transfer of inoculum is obligatory and essential for completing the life cycle. So barberry called the alternate host.
- Similarly, *Cronartium ribicola* which causes blister rust of the white pine also attacks wild or cultivated currant or gooseberry plants as its alternate host,
- Cedar is an alternate host of *Gymnosporangium juniperi-virginianae* causing cedar- apple rust.
- Both *Rhizoctonia solani* and *Sclerotium roilfsii* have very wide host range spread over many families. As a result the inoculum of these pathogens is available almost throughout the year for infecting the same or different host species.

Alternative hosts

Such transfer of inoculum from one host species to another is not compulsory. There are called **alternative hosts.**

- Cereal rusts can infect their wild hosts and survive the winter crop periods in such hosts and also some self sown wheat plants at higher altitudes.
- Powdery mildew fungus *Erysiphe cichoracearum* and viral pathogens of cucurbits also survive the inter-crop period in wild cucurbits when their normal crop host is absent.
- Important rice pathogens, *Helminthosporium oryzae* and *Pyricularia oryzae* can survive through their weed hosts like *Leersia heaxandra*, *Echinochloa colonum*, and *Setaria intermediata* and *Digitaria marginata* y or some others.

Vectors as hosts

- Among fungi and bacteria, which are transmitted by insects, some over winter within the body of their vectors, e.g., *Ceratocystis fagacearum* (oak wilt) survives in nitidulid beetles, *Xanthomonas campestris* pv. *stewartii* (maize wilt) in flea beetles and rice stunt virus in leaf hoppers.

Infected seed or planting material

- Infected or contaminated seeds and other planting material may carry the inoculum of fungal, bacterial, viral or nematode pathogens through the inter-crop period or the unfavourable season.
- Some attack the floral organs and enter in to the ovary and others infect the seed through the seed coat.
- Some infect the embryo and others the tissue beneath the seed coat and remain there in a dormant state without causing any damage to the seed and affecting their germinability.
- *Ustilago tritici* and *Alternaria triticina* (in wheat), *Alternaria brasscicola* (in brassicas) survive in/on the seed.
- *Phytophthroa infestans* (causing late blight of potato), *Peronospora destructor* (downy mildew of onion), *Pythium aphanidermatum* (rhizome rot of ginger), red rot

of sugarcane, leaf roll and mild mosaic viruses of potatoes survive in the planting material like tubers, bulbs, rhizomes etc of the respective crops.

- Pathogens surviving in or on the seed or vegetative planting material are already in contact with the potential host plants and are not exposed to the vagaries of the environment.
- When the seeds germinate, the pathogens also become active, resume their growth or infect the seedlings at the earliest opportunity.
- Some other pathogens which enter through the seed coat and do not affect the embryo are *Septoria apicola* in celery, *Colletotrichum* in chillies, *Pseudomonas phaseolicola* in bean and *Clavibacter michiganese* in tomato.
- Seeds may also get contaminated with the pathogen during threshing and storage. For example, covered smut of barely (*Ustilago hordei*), grain smut of jowar (*S. sorghi*), bunt of wheat (*Tilletia caries* and *T. foetida*) or fruit rot of chillies (*Colletotrichum capsici*).
- Longevity of fungal spores carried externally on seed varies considerably for different pathogens depending upon storage conditions.
- While thin walled spores perish early, those with thick walls survive longer.

Crop Residue

- Crop residue consisting of infected plant parts (leaves, stems, fruits etc.), roots and stubbles help in the survival of plant pathogens are a major source of primary inoculum for the next crop to be grown there.
- Examples are *Colletotrichum falcatum* in sugarcane, *Cercosporidium personatum* in groundnut, *Alternaria brassicae* in crucifers, *Ascochyta pisi* in pea, *Xanthomonas campestris* pv. *campestris* in cabbage, *Xanthomonas campestris* pv. *oryzae* in rice, bean mosaic virus in bean and barley mosaic virus in barely.

Soil

- Soil is the ultimate resting place for most of the plant pathogens and one of the major source of primary inoculum for fungal, bacterial or nematode pathogens.
- Most pathogens come back to soil after completing parasitic existence on the host plants. Some of them survive in soil as free living saprophytes and also reproduce and complete their life cycles.

- Others just survive there showing only limited growth and no reproduction. Those that attack aerial organs of the hosts mostly survive the inter crop period or adverse climates through resistant propagules.
- Garrett (1950) distinguished behavior of soil borne fungal pathogens and grouped them as soil inhabiting and soil invading fungi.
- **Soil inhabiting fungi** were characterized by their ability to survive indefinitely in soil as saprophytes and to complete their life cycles there, e.g., *Pythium*, *Phytophthora, Rhizoctonia* sp., *Sclerotium rolfsii* and *Fusarium solani.* They are mostly primitive, less specialized parasites.
- **Soil invading fungi** also known as **root inhabiting fungi** were characterized by an extended parasitic phase on living host plants, mostly restricted to host roots and a declining saprophytic phase in soil after death of the host plant.
- The root inhabiting fungi with characteristic local distribution in soil are: *Gaeumannomyces graminis, Fomes annosus*, *Phymatotrichum omnivorum* and *Armillariella mellea.*
- Vascular pathogens including *Verticillium albo-atrum* represent the intermediate group. They are no doubt somewhat specialized in their parasitic habits but can survive also in soil saprophytically for a few years. Their ability to form perennating structures like chlamydospores (in *Fusarium oxysporum*) or micro sclerotia (*Verticillium albo-atrum*) may have a contributory role in this respect.

Resting structures

- Many soil borne fungal pathogens survive effectively in soil through adverse circumstances by forming resting structures.
- Basically, the resting structures are of two types:

i) resting spores of sexual or asexual origin

ii) resting organs made of vegetative hyphae.

- Thick-walled chlamydospores help in the survival of many fungi as *Pythium* spp., *Fusarium solani* and *F. oxysporum.*
- Resting spores of *Plasmodiophora brassicae* also act in perennation. The conidia of *Diplocarpon rosae* also participate in the act of survival.

- Smut spores some times also called as chlamydospores also function in survival of some rust, and smut and bunt fungi, termed respectively as teleutospores, smut spores and bunt spores.
- Some fungi form sclerotia which are black hardened structures made up of aggregations of vegetative hyphae and are able to survive through extremely unfavourable conditions.
- *Sclerotium rolfsii, Rhizoctonia* spp. and *Phymatotrichum omnivorum* are some of the common fungal pathogens with an important role for sclerotia in their survival.

Lecture 22

EFFECT OF ENVIRONMENTAL FACTORS ON DISEASE DEVELOPMENT

Aim: To acquaint the students with effect of environmental factors on disease development

Effect of Environmental Factors on Disease Development

- Plant diseases are ubiquitous throughout the world wherever plants grow, but of more common occurrence in humid to wet area with cool, warm or tropical temperatures.
- Diseases most commonly occur during wet, warm days and night and on plants heavily fertilized with nitrogenous fertilizers.
- So, environmental conditions frequently determine whether a particular disease will occur or not.
- Most common environmental factors that have considerable influence on development of plant disease are temperature and moisture.
- Other factors include wind, light, soil pH, soil structure etc.

Effect of temperature

- Each pathogen has an optimum temperature for its growth.
- Different growth stages of fungus, such as the production of spores, their germination and the growth of the mycelium may have slightly different optimum temperature.
- Storage temperatures for certain fruits, vegetables and nursery stock are manipulated to control fungi and bacteria that causes storage decay, provided the temperature does not change quality of products.
- In temperate regions, low temperature during late fall, winter or early spring are not congenial for the development of pathogen, but as the temperature rises, these pathogens become active and when other conditions are favourable they can cause infection and thus disease.
- Pathogen differs in their preference for higher or lower temperature. For example, the fungi namely *Typhula* and *Fusarium* causing snow mould of cereals and turf grasses, late

blight pathogen *Phytophthora infestans* are more serious in cold regions whereas fungus like *Colletotrichum, Ralstonia* are favoured by higher temperature.

- Rapid disease development occurs when temperature is optimum for pathogen development and is below or above the optimum for host development.
- For stem rust of wheat (*Puccinia graminis tritici*) completion of infection cycle is 22 days at 5°C, 15 days at 10°C and 5-6 days at 23°C.
- The minimum, optimum and maximum temperature for the pathogen, host and disease are same, the effect of temperatures in disease development is apparently through its influence on pathogen.
- Effects of temperature may mask symptoms of certain viral and mycoplasmal diseases and making them more difficult to detect.

Effect of moisture

- Moisture influences the initiation and development of infectious plant diseases in many interrelated ways.
- It may exist as rain or irrigation water on plant surface or around the roots, as relative humidity in the air and as dew.
- Moisture is indispensable for the germination of fungal spores and penetration of the host by germ tube.
- It is also indispensable for the activation of bacterial, fungal and nematode pathogens before they can infect the plant.
- Moisture in the form of splashing rain and running water also plats an important role in the distribution and spread of many of the pathogens on the same plant and on other plants.
- Moisture also increases the succulence of host plants and thus their susceptibility to certain pathogens, which affects the extent and severity of disease.

Effect of rainfall

- The occurrence of many diseases in a particular region is closely correlated with the amount and distribution of rainfall within year.
- Late blight of potato, apple scab, downy mildew of grapes and fire blight are found or are severe only in areas with high rainfall or high relative humidity during the growing season.
- In apple scab, continuous wetting of the leaves, fruits etc. for at least 9 hours is required for primary infection to take place even at optimum range (18 to 23°C) of temperature.
- At lower temperature the minimum wetting period required is higher.
- In powdery mildews, spore germination and infection are actually lower in the presence of free moisture on the plant surface than they are in its absence.

Effect of Relative humidity

- Relative humidity is very critical in fungal spore germination and the development of storage rots.
- Rhizopus soft rot of sweet potato (*Rhizopus stolonifer*) is an example of storage disease that does not develop if relative humidity is maintained at 85-90 %, even if the storage temperature is optimum for the growth of the pathogen. Under these conditions, the sweet potato root produces corky tissues that wall off the *Rhizopus* fungus.
- Moisture is generally needed for fungal spore germination, the multiplication and penetration of bacteria and the initiation of infection e.g., germination of powdery mildew spores occurs at 90-95 % relative humidity.

Effect of soil moisture

- Soil moisture influences the initiation and development of infectious plant diseases.
- High or low soil moisture may be a limiting factor in the development of certain root rot diseases.
- High soil moisture levels favours development of destructive water mould fungi, such as species of *Aphanomyces, Pythium* and *Phytophthora.*

- Overwintering by decreasing oxygen and raising carbon-dioxide levels in the soil makes roots more susceptible to root rotting organisms.
- Diseases such as take all of cereals (*Gaeumannomyces graminis*), charcoal rot of corn, sorghum and soyabean (*Macrophomina phaseolina*), common scab of potato (*Streptomyces scabies*) and onion white rot (*Sclerotium cepivorum*) are most severe under low moisture levels.

Effect of wind

- Most plant diseases that occurs in epidemic portions and spread in large areas are caused by fungi, bacteria and viruses that are spread either directly by wind or indirectly by insects which can travel long distances with the wind.
- Uredospores and many conidia are transported to many kilometers by wind.
- Wind becomes more important when it is accompanied by rain.
- Wind blown rain splashes can help in spread of bacteria from the infected tissues.

Effect of light

- Light intensity and duration may either increase or decrease the susceptibility of plants to infection and also the severity of disease.
- Light mainly cause production of etiolated plants due to reduced light intensity which in turn increases the susceptibility of plants to non-obligate parasites but decreases the susceptibility of plants to obligate parasites.
- It also enhances the plants' susceptibility to viral infections.

Effect of soil pH

- Soil pH is a measure of acidity or alkalinity and it markedly influences occurrence of soil borne pathogens.
- Growth of potato scab (*Streptomyces scabies*) pathogen is suppressed at a pH of 5.2 or slightly below but is more severe at a pH 5.2 to 8.0 or above.
- Club root of crucifers caused by *Plasmodiophora brassicae* is most severe at a pH of 5.7, whereas its development drops sharply between 5.7 and 6.2 and is completely checked at pH 7.8.

Effect of soil type

- Certain pathogens are favored by loam soils and others by clay soils.
- Fusarium wilt disease which attacks a wide range of cultivated plants causes more damage in lighter and higher soils.
- Nematodes are also most damaging in lighter soils that warm up quickly.

Effect of host-plant nutrition

- Nutrition affects the rate of growth and the state of readiness of plants to defend them against pathogenic attack.
- Nitrogen abundance results in the production of young, succulent growth, a prolonged vegetative period and delayed maturity of the plants.
- These effects make the plant more susceptible to pathogens that normally attack such tissues and for longer periods.
- In contrast, plants suffering from a lack of nitrogen are weaker, slow growing and faster aging.
- Such plants are susceptible to pathogens that are best able to attack weak, slow-growing plants.
- Large amounts of nitrogen increases the susceptibility of pear to fire blight (*Erwinia amylovora*), wheat rust (*Puccinia*) and powdery mildew (*Erysiphe*).
- Reduced availability of nitrogen may increase the susceptibility of tomato to Fusarium wilt and *Alternaria solani*, of sugar-beets to *Sclerotium rolfsii* and of most seedlings to Pythium damping-off.
- Severity of the disease caused by *Fusarium* spp., *Plasmodiophora brassicae* and *Sclerotium rolfsii* increases when an ammonium fertilizer is applied whereas the severity of diseases caused by *Streptomyces scabies* and *Gaeumannomyces graminis* increase when nitrate form of fertilizers are applied.
- Phosphorus has been shown to reduce the severity of potato scab but to increase the severity of cucumber mosaic virus on spinach and *Septoria* infection of wheat leaves and glumes.

- Phosphorus seems to increase resistance either by improving the balance of nutrients in the plant or by accelerating the maturity of the crop and allowing it to escape infection by pathogens that prefer younger tissues.

- Potassium has also been shown to reduce the severity of numerous diseases including stem rust of wheat and early blight of tomato, whereas high amounts of potassium increase the severity of rice blast and root knot.

- Potassium seems to have a direct effect on the various stages of pathogen establishment and development in the host and an indirect effect on infection by promoting wound healing.

- Calcium reduces the severity of several diseases caused by root and stem pathogens such as *Rhizoctonia, Sclerotium, Botrytis* and *Fusarium oxysporum,* but it increases the severity of common scab of potato (*Streptomyces scabies).*

- The effect of calcium on disease resistance seems to result from its effect on the composition of cell walls and their resistance to penetration by pathogens.

- In general, plants receiving a balanced nutrition, in which all required elements are supplied in appropriate amounts, are more capable of protecting them from new infections and of limiting existing infections than plants to which one or more nutrients are supplied in excessive or deficient amounts.

Effect of pollutants

- Air pollutants cause various types of direct symptoms on plants exposed to their high levels.

- Ozone may affect a pathogen and sometimes the disease it causes. For example in wheat rust fungus, ozone reduces the growth of uredia and of hyphal growth and also the number of uredospores produced on ozone injured leaves.

- Ozone increases the infection of potato leaves by *Botrytis*.

Lecture 23

PLANT DISEASE EPIDEMIOLOGY

Aim: To acquaint the students with plant disease epidemiology

- Epidemiology deals with the outbreaks and spread of diseases in a population.
- It is the study of rate of multiplication of a pathogen which determines its capacity to spread a disease in a plant population.
- It is the most important part of the study of plant diseases from practical point of view.
- Epidemiologically, the diseases have been described as

  i) Simple interest diseases

  ii) Compound interest diseases.

Simple interest diseases

- In these diseases, the rate of increase is mathematically analogous to the simple interest in money.
- There is only one generation of the pathogen in the life of the infected crop.
- The primary inoculum is seed or soil borne and secondary infection is rare.
- All the infections noticed in the field are from the pre-existing inoculum in the soil.
- Most important example is loose smut of wheat (*U. tritici*), where the inoculum is internally seed borne or is carried in the seed resulting in the infected ears in the season showing black powdery loose mass in place of grains.
- In wilt and root rot diseases, the primary inoculum is important and there are very remote chances of secondary spread, even if the pathogen sporulates, due to the soil barriers and other factors.

Compound interest diseases

- In these diseases, the rate of increase is mathematically analogous to compound interest in money.
- The pathogen produces spores at a very rapid rate which are disseminated by external agencies like air, thereby infecting other plants.
- The incubation and sporulation period is also very short.
- The infection cycle is repeated many times during the cropping season.
- Secondary inoculum plays a vital role in the development of epidemics in such diseases.

- The prevailing environmental conditions play an important role in such diseases.
- Wheat rust, late blight of potato and apple scab are some diseases of this type.

Mathematical model of disease spread

- Vander Plank in 1963 in his historical book "Plant Diseases- Epidemics and Control" suggested a model based on the infection rate 'r'.
- It is the rate at which the population of the pathogen increases. The 'r' is on average estimated from successive estimates of population of pathogen as proportion 'X' of the infected plants (in case of systemic diseases) or of infected susceptible tissues in case of local lesion diseases.
- The equation for describing the epidemic is:
- $X = X_0^{ert}$

Where X	=	proportion of disease at any time
X_0	=	the amount of critical inoculum
r	=	average infection rate
t	=	time during which infection occurred

- The value of e in the equation is the base of natural logarithm = $\left(1+\frac{1}{n}\right)^n$
- We are dealing here with an exponential function and the basic assumption here is that at a given time the rate of disease increase is proportional to the amount of disease at that moment. This assumption is true at the beginning where plenty of tissue is there to be infected. As the disease progresses, the amount of susceptible tissue left for infection declines and the rate of increase is determined by the amount of susceptible tissue left i.e. 1-x, but not the one which is present (x). The infection rate 'r' is very important and used to compare epidemics of diseases at different localities/cultivars /fungicide treatments. The comparative 'r' is derived by taking log and transposing:
- $\log e^{x} = \log e^{x0} + rt$
- $rt = \log e^{x} - \log e^{x0}$
- $r = \frac{1}{t} \log e^{x/x0}$
- This 'r' can be assumed at any time during the epidemic but its use is simpler in early stages of development of the epidemics which is an important stage. For comparing two epidemics in cultivars or otherwise, 2 readings at the starting point i.e. t_1 (x_1) and the other time i.e. t_2 (x_2) are must.

- Average infection rate in them calculated as:

$$r = \frac{1}{t_2 - t_1} \log e \frac{x_2}{x_1}$$

- When the disease has reached very high intensity, the formula for calculating 'r' is more complicated because the factor (1-x) is introduced. In that case:

$$R = \frac{1}{T_2 - t_1} \log e \frac{x_2 (1-x)}{x_1 (1-x)}$$

Slow and rapid epiphytotics

- The form an epidemic can take is governed by
- the nature of pathogen
- its host
- the weather which works as a reference in the battle between the two.
- At one extreme are the epidemics which develop slowly (tardive) and at the other end are those which develop rapidly (explosive).
- Many intermediate types may also occur.
- Slow epiphytotics occur among populations of perennial, long lived plants such as fruit trees.
- The causal organisms are mostly systemic to varying extents e.g. Dutch elm disease or chestnut blight.
- Rapid epidemics are chiefly caused by non-systemic pathogens with high multiplication/ reproduction rate which have several generations within a short time.
- Mostly annual crops are affected by such epiphytotics.
- They are more affected by environments than the slow epiphytotics.
- The increase of disease is rapid rising to distinct peak in short time, then showing sharp decline as the weather turns unfavorable, host become resistant due to maturity etc.

Analysis of epidemics

- Epidemic is a system i.e. an inter-locking complex of processes characterized by many reciprocal cause- effect pathways.
- It is a structural and functional phenomenon having two or more separable components and some interaction between these components.

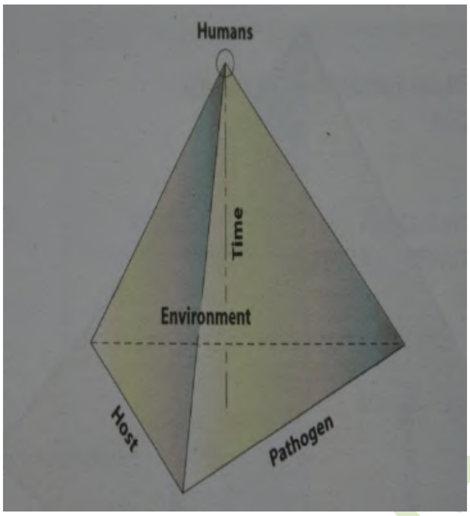
- However, each component of the system can be studied separately.
- Analysis of a system with 2 or a few components is easy but that with too many variable and non variable components with their own components and all interacting with each other is a tedious and time consuming job.
- Epidemics interact with other sub eco-systems of agro-ecosystem making it more complex.
- In such a complex scenario, it is rather difficult to pin point the individual effect of any of the parameters say temperature or rainfall or any cultural practice on development of epidemics and its overall contribution.
- However, there are some diseases in which the role of some critical parameters like temperature, leaf wetness and rainfall or RH has been studied.

Elements of epidemics

- The 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,
- favourable environmental conditions over a relatively long period of time.
- Humans may unwittingly help initiate and develop epidemics through some of their activities, e.g., by tipping or pruning plants in wet weather.
- Humans may also stop the initiation and development of epidemics by using appropriate control measures under situations favourable for epidemics.
- The chance of an epidemic increases when the susceptibility of the host and virulence of the pathogen are greater, as the environmental conditions approach the optimum level for pathogen growth, reproduction, and spread, and as the duration of all favourable combinations is prolonged or repeated.

The Disease Pyramid

- To describe the interaction of the components of plant disease epidemics, the disease triangle can be expanded to include time and humans factors.
- The specific point in time at which a particular event in disease development occurs and the length of time during which the event takes place affect the amount of disease.
- The interaction of four components can be visualized as a tetrahedron, or pyramid, in which each plane represents one of the components. This figure is referred to as the **disease tetrahedron** or **disease pyramid**.



Disease tetrahedron or **disease pyramid**.

- The effect of time on disease development becomes apparent when one considers the importance of the time of year, the duration and frequency of favourable temperature and rains, the time of appearance of the vector, the duration of the infection cycle of a particular disease, and so on.
- **If the four components of the disease tetrahedron could be quantified, the volume of the tetrahedron would be proportional to the amount of disease on a plant or in a plant population.**

Effect of Humans

- Disease development in cultivated plants is also influenced greatly by a fifth component: humans.
- 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.
- By the resistance of the particular plants they cultivate, humans also determine which pathogens and pathogen races will predominate.
- By their cultural practices, and by the chemical and biological controls they may use, humans affect the amount of primary and secondary inoculum available to attack plants.
- Humans also modify the effect of environment on disease development by delaying or speeding up planting or harvesting, by planting in raised beds by protecting plant surfaces with chemicals before rains, by regulating the humidity in produce storage areas, and so on.

- The timing of human activities in growing and protecting plants may affect various combinations of these components to a considerable degree, thereby affecting the amount of disease in individual plants and in plant populations greatly.
- The human component has sometimes been used in place of the component “time” in the disease tetrahedron, but it should be considered a distinct fifth component that influences the development of plant disease directly and indirectly.

Lecture 24

PLANT DISEASE FORECASTING

Aim: To acquaint the students with plant disease forecasting methods

Plant disease forecasting

Plant disease forecasting involves all the activities in ascertaining and notifying the farmer in an area/community that the conditions are sufficiently favourable for certain diseases, that application of control measures will result in economic gain or that the amount of disease expected is unlikely to be enough to justify the expenditure of time, money and energy for its control.

- Plant disease forecasting requires complete knowledge of epidemiology i.e. the development of disease in plant population under the influence of the factors associated with the host, the pathogen and the environment.
- Forecasting is actually, the applied epidemiology.
- Plant disease forecasting is made more reliable if the reasons for a particular disease developing under certain conditions and not others are known.
- Experimental investigation is necessary to show that exactly what stage during the disease development is critical for variable incidence or intensity of disease.
- A timely and reliable forecast gives the farmer many options to choose from that he can weigh the risks, costs and benefits of his possible decisions.

Requirements or conditions of plant disease forecasting

- The disease must be causing economically significant damage in terms of loss of quantity and quality of the produce in the area concerned.
- The onset, speed of spread and destructiveness of the disease is variable mostly due to dependence on the weather which is variable.
- Control measures are known and can be economically applied by the farmer when told to so.
- Information on weather- disease relationship is fully known.

Methods of forecasting plant diseases

Plant disease forecasting has been applied in many diseases on the basis of:

- Weather conditions during the inter-crop period
- Weather during crop season
- Amount of disease in the young crop

- Amount of inoculum in the air, soil or planting material

Forecasts based on weather conditions during inter-crop period and amount of primary inoculum

- Weather during inter-crop period is closely related to the survival of many plant pathogens, mostly through the severe cold of winter months and in some cases to that of pathogen vectors also.
- *Erwinia stewartii* (causing Stewart wilt of corn) survives the winter in the bodies of flea beetles- its vectors.
- An assessment of vector population at the onset of spring gives an indication of the extent of survival of the vector through the cold months.
- Where the sum of mean temperatures for 3 winter months of December, January and February at a given location is less than -1^{o}C, the most of vectors die and therefore no serious disease is expected.
- In contrast, mild winter permits good survival of vectors leading to severe disease outbreak. For curly top disease of sugar beet, severity of disease outbreak is correlated with the number of vectors that successfully overwinter.
- In California, USA, fire blight of apple and pear incited by *Erwinia amylovora*, became severe if the daily average temperature exceeded a 'disease prediction line' obtained by drawing a line from 16.7^{o}C on March, 1 to 14.4^{o}C on May, 1. This pathogen multiplies very slowly at temperatures below 15^{o}C, and this makes the initial inoculum inadequate for any strong attack.
- Some fungal pathogens affecting temperate region crops like those causing apple scab, brown rot, ergot of rye, etc. survive winter by developing resistant structures. The forecasters look for them to get hints about their prospects in the coming season.
- For some soil borne fungal pathogens like *Verticillium* and *Sclerotium* spp. and cyst nematodes *Heterodera* and *Globodera* spp., greater the amount of propagules (sclerotia and cysts, respectively) more severe is the disease.

Weather conditions during the crop season and the production of secondary incoculum

- The temperature and moisture levels are very critical during the crop season for the development and spread of some air borne diseases.
- Severe outbreaks are likely to occur if certain combinations of temperature and moisture levels are available for a certain period of time.
- This requirement is different for different diseases.
- Several leaf spot diseases of fungal origin for example tikka disease of groundnut, turcicum blight of corn, apple scab and paddy blast can be predicated by taking into

account the number of spores trapped daily over the cultivated field, the temperature and relative humidity over a certain period of time.

Amount of disease in the young crop

- Extent of disease developed in the young crop may occasionally provide a reliable indication of the likely severe development of the disease in the mature crop, e.g., leaf rust of wheat.
- The young crop mostly gets infected from overwintering local infections.
- The amount of infection at onset of spring often determines the subsequent development of this disease as the weather following is generally favourable.
- The crop is, therefore, periodically assessed to keep track of the infection and control measures are recommended accordingly.

Amount of inoculum in soil, planting material and air

- In many diseases, the primary inoculum comes from last years infected crop residues lying in the field.
- Overwintering fungal pathogens produce spores at the onset of the growing season which function as primary inoculum and initiate initial infections in some plants of the new crop.
- Examples are apple scab, brown rot of stone fruits or ergot of rye.
- The amount of such residues lying in the field or the plantation floor gives an indication of the availability of inoculum at the start of the season and if the level is high a forecast can be made.

Amount of inoculum in soil, planting material and air

- In the seed-borne plant diseases, the extent of infection and contamination in the seeds or planting material can be easily estimated in the laboratory.
- This can be useful for predicting seed-borne smut, bacterial and viral diseases.
- Depending on the level of infection or contamination, the seed or planting material can be rejected out rightly or made free from the pathogens by chemical or thermal treatments.
- Some soil borne fungal pathogens may survive in soil as hyphae; but most of them survive in soil as resting structures like sclerotia, chlamydospores and stromata.
- The soil borne inoculum can be approximately determined and if exceeds certain limits, a susceptible variety may not be grown in such fields.
- Blight and root rots caused by *Sclerotium rolfsii* are good examples.

Some successful examples of plant disease forecasting

Late blight of potato

- Holland pioneered the development of forecasting and spray warning services for the control of late blight of potato.
- Van Everdingen (1926) analyzed the combined effect of several weather conditions on the development of *Phytophthora infestans* and evolved four rules, popularly known as **Dutch rules,** on which the appearance of blight was observed to depend.

  i) Night temperature below dew point at least for 4 hours

  ii) A minimum temperature of 10^{o}C or above

  iii) A mean cloudiness on the next day of 0.8 or more, and

  iv) At least 0.1mm rainfall during the next 24 hours.

- When all these four conditions were obtained in Holland, potato blight was expected after the next 7 days and therefore the control measures were immediately recommended to the farmers.
- Beaumont and Hodson (1930) added the fifth rule to above four rules to apply them in England. The additional rule called for periods of not less than 2 days in which the relative humidity (at 3 PM) was higher than 75 percent.
- Staniland (1937) devised a system known as Beaumont period and reduced these five rules to two, according to which when a minimum temperature of 10^{o}C or over and a relative humidity 75 per cent were available, the situation was right for forecast.
- The Beaumont period was later replaced by Smith period (Smith, 1961) which comprised the minimum temperature of 10^{o}C for two consecutive '24 hour periods' in each of which there was at least 11 hours with more than 89 per cent relative humidity; and its fulfillment prompted forecast.
- Krause *et al.* (1975) devised for USA a complete forecasting system known as BLITECAST.
- It is a computer programme written in Fortran IV. In this system, local temperature, relative humidity and rainfall data is fed in to the centrally based computer system, which then provides the forecast.
- Later, addition of data on cultivar resistance or fungicide to be used corrected the forecast and made it more appropriate.
- In India, fungicide application is recommended when temperature is lower than normal and relative humidity is close to saturation point.

Apple scab forecasting

- In case of apple scab caused by an ascomycete *Venturia inaequalis*, the peudothecia present in the stromata embedded in the over-wintered infected leaf litter on the orchard floor start maturing in the spring.
- Ascospores are discharged from them continuously for 3-5 weeks and function as primary inoculum.
- Being air borne, ascospores land on young expanding apple leaves and start primary infections.
- Mills and La Plante (1954) developed a Mills' Chart according to which the primary infection could occur within a temperature range of 0 to 28^{o}C and leaf wetness for 9 to 48 hours.
- A Mills' period was defined as a period of leaf wetness required at a particular temperature for causing light, medium or heavy scab infection on the leaves.
- By combining the temperature and duration of leaf wetness for varying periods, they suggested that combination of 30, 20, 14 and 10 hours of leaf wetness, respectively with a mean temperature of that period of 5.6, 7.2, 10 and 15^{0}C satisfied the requirements for successful infection among others.
- It also indicated the expected severity of disease along with incubation period. Later, Smith (1961) utilized a threshold level of 90% relative humidity following rain in place of leaf wetness record.
- Jones *et al.* (1981) and Ellis *et al.* (1984) devised computer based apple scab predictor, which utilized special purpose micro-processor for analyzing detailed information on various relevant components.
- Recommendations were made for need based timely fungicide application and even the type of fungicides to be used.

Apple scab forecasting in Himachal Pradesh

- In Himachal Pradesh, when the apple scab appeared in epidemic form in late 1970's onward, 5 Apple Scab Monitoring and Research Laboratories were established at Mashobra, Kotkhai, Thanedhar and Sarahan in Shimla, and Bajaura in Kullu district.
- Apple scab infection periods were predicted by using equipments, like de Wit 7 day leaf wetness recorder, Reuter Stokes apple scab predictor and SWG Biomat at different locations.
- When the conditions congenial for the development of apple scab were recorded, this information was given to the farmers by blowing sirens, radio broadcasts and personal contacts.

- Of all the systems, Reuter Stokes predictor was found to be the most efficient in predicting the infection periods at Kotkhai (Sharma and Gupta, 1995).
- Different fungicides could be effectively used depending on their after-infection (curative), pre-symptom or post-symptom (eradicative) activities. This is regarded as the best example of plant disease forecasting in India till date.

Lecture 25

MEASUREMENT OF PLANT DISEASES AND YIELD LOSS

Aim: To acquaint the students with measurement of plant diseases and yield loss

Measurement of plant diseases

Plant diseases are measured in terms of incidence and severity.

- **Disease incidence** is the number or proportions of plant units that are diseased (i.e. plants, leaves, flowers, fruits etc.) in relation to the total number of the units examined.

It is expressed in terms of percentage as follows:

$$\text{Per cent disease incidence} = \frac{\text{Total number of plants/units diseased}}{\text{Total number of plants/units examined}} \times 100$$

The measurement of disease incidence is relatively quick and easy and is used to measure/assess a disease through a field, region or country.

- **Disease severity**

- In a few cases such as cereal smuts, neck blast, brown rot of stone fruits and vascular wilts of annuals where they cause total loss, disease incidence has a distinct relationship with the severity of the disease and yield loss.
- **Disease severity** is usually expressed as the proportion of plant area or fruit volume destroyed by the pathogen. It is expressed in the percentage as follows:

$$\text{Per cent disease severity} = \frac{\text{Total amount of plant/plant part area destroyed}}{\text{Total number of plant/plant part area examined}} \times 100$$

- In many diseases such as most leaf spots, root lesions and rusts in which plants are counted as diseased whether they are exhibiting a single lesion or hundred lesions, disease severity is of greater importance to the growers than the disease incidence.
- Disease severity is also referred to as disease intensity.
- For diseases, where the amount of disease varies greatly on different plants in the population, many arbitrary indices and ratings have been in practice.
- They are usually discouraged and are replaced by percentage scales and standard area diagrams of disease intensity.

Percentage Scales

- In this, usually the number of plants or organs falling into known percentage disease groups are recorded.
- The disease groups are the categories distinguished on the basis of per cent damage seen by human eye.
- A 12 grade scale was suggested by Horsfall and Baratt (1945) who took into the fact that the grades detected by the human eye are approximately equal divisions on a logarithmic scale and generally follow the Weber-Fechner law which states that visual activity depends on the logarithm of the intensity of the stimulus.
- In percentage disease assessment, the eye actually assesses the diseased area upto 50 per cent and the healthy area above 50 per cent.

In **Horsfall and Baratt grading system**, the categories were as follows:

1= 0%, 2= 0-3%, 3= 3-6%, 4= 6-12%, 5= 12-25%, 6= 25-50%, 7= 50-75%, 8 = 75-87%,

9 = 87-94 %, 10= 94-97%, 11= 97-100 %, 12 =100% disease.

- This is a logarithmic scale and is satisfactory not only for disease measurement, but also for epidemiological studies, because pathogens multiply at logarithmic rate and also for loss appraisal.
- A system using percentage scale was developed by British mycological Society (Anon, 1947) for measuring late blight of potato.

The percentage scales have many advantages such as:

i) The upper and lower limits of the scale are always well defined.

ii) The scale is flexible in that it can be divided and subdivided conveniently.

iii) It is universally known and can be used to record both the number of plants infected (incidence) and area damaged (severity) by a foliage or root pathogen.

Standard area Diagrams

- Nathan Cobb developed the first standard area diagram for leaf rust of wheat.
- It divided rust intensity into five grades representing 0, 5, 10, 20 and 50 per cent of leaf area occupied by the visible or sporulating rust pustules.
- The highest grade (50%) represented the maximum possible cover.
- A modified Cobb's scale was proposed by Melchers and Parleu (1922) for the estimation of stem rust of wheat.

- One of the most practical set of the area diagrams was prepared by James (1971). These diagrams represent the actual area of the leaves, stems, pods and tubers occupied by lesions in terms of per cent area covered.

- A common formula as follows is generally used to calculate the average infection or Infection Index, sometimes also known as Disease Index or Per cent Disease Index, which is calculated as follows:

Per cent Disease Index (PDI)	=	Sum of all disease ratings	X	100
		Total number of ratings	X	Maximum disease grade

- Severity estimates from fairly small areas can be combined to cover large areas,viz., village, district or state. This overall index can be obtained by using the formula:

Percent Disease Index (PDI)	=	Field rating class	X	Number of hectares in the class
		Total number of hectares		

Remote sensing

- Remote sensing provides a powerful tool for detection and measurement of diseases.
- It is used for survey of large crop areas by means of aerial photography.
- Diseased plants can be identified as distinct patches in an otherwise uniform picture.
- It is useful for measuring diseases such as late blight of potato where early disease foci can be located and their subsequent spread is followed.
- Remote sensing is also useful when sudden disaster strikes and a rapid appraisal of the situation is required.
- In wheat crop, aerial photography can be used to determine the extent of damage by take-all or eye spot disease.
- Similarly, the incidence of *Heterobasidion* can be monitored in pine plantation covering undulating, boggy or rocky land.

Crop loss assessment

Crop loss can be summarized as the difference between the attainable yield from the healthy crop and that obtained from the diseased crop and is expressed as percentage mostly in terms of money.

- The most important purpose of disease appraisal is the assessment of crop loss.
- Various attempts have been made to utilize disease assessment data for estimation of loss.
- However, such conversion is not easy.

- There is no straight forward way to determine the amount of yield loss.
- While calculating the yield loss from a disease, its nature, extent of damage in terms of yield, quality and loss of market value are to be considered.
- Diseases like smuts, root rots, ergot etc. cause almost 100 per cent damage to the crop and the loss estimates are rather easy to make.
- However, those causing damage to the foliage and other debilitating diseases, thereby affecting the yield partially to different extents pose a great difficulty in assessing losses.
- Sometimes, the crop stage, when the crop is attacked becomes critical in this respect.

Crop Loss (%)	=	Attainable yield/income from the healthy crop-Obtained yield/income from the diseased crop	X	100
		Attainable yield/income from the healthy crop		

Estimation of yield loss

- For estimating yield loss due to diseases, comparisons between crops grown in different years or localities are not reliable as other factors are not the same.
- For valid comparisons, disease free plots are to be compared with those nearby with varying amount of disease.
- Disease free plots are mostly obtained by use of fungicides with little or no phytotoxicity.
- If the yield loss is to be estimated on a regional basis, data on disease incidence obtained from the disease surveys would be utilized using formulae based on fungicide trials.
- These data are usually employed in the 'critical point' models which are actually the regression equations.
- 'Multiple point' models in which the loss estimation is based on many diseases appear to be more reliable.
- These data are used to produce a multi-dimensional model whose dimensions include the date of disease onset, shape of the disease progress curve, the host cultivar and the yield loss as the dependent variables.

Lecture 26

PRINCIPLES OF PLANT DISEASE MANAGEMENT

Aim: To acquaint the students with general principles of plant disease management

Fundamental principles of disease management

i) Avoidance: Geographical area, selection of a proper field, planting time and disease escaping varieties, avoidance of insect vectors and wed hosts

ii) Exclusion: Quarantine, inspection & certification, seed treatment

iii) Eradication: Crop rotation, sanitation, rogouing, soil treatment, heat and chemical treatment to diseased plant material, use of antagonists

iv) Protection: Chemical treatment

v) Immunization: Resistant varieties, induced systemic resistance

vi) Therapy: Chemotherapy, thermotherapy

Avoidance

- It involves tactics that prevent contact between the host and the pathogen.
- The selection of geographic area, selection of a proper field, planting time and disease escaping varieties play an important role in avoiding the disease.
- For example bean anthracnose is common in wet areas. Similarly smut and ergot of pearl-millet are serious in areas where rainfall occurs for long durations during flowering of the crop.
- Successful cultivation of a crop depends to a great extent on the selection of a proper field especially in soil borne diseases, e.g., root knot nematode disease, wilt of pigeon-pea etc.
- In many diseases the incidence or disease severity depends upon the coincidence of susceptible stage of the host and favourable conditions for the pathogen.
- This can be achieved by alteration in the date of planting/sowing.

- Certain insects especially aphids, beetles and leafhoppers are known to transmit viruses and mollicutes from infected plants to healthy plants.
- Perennial weeds including pokeweed, milkweed, Johnson grass and horse nettle serve as over-wintering reservoirs of some viruses.
- Curly top in sugar-beet is a leaf hopper-transmissible viral disease and weeds play a significant role in its spread.
- Some of the important weeds involved in the spread of curly top disease are certain species of *Chenopodium*, Russian thistle, *Amaranthus*, deadly nigh shade, shepherd's purse and knotweed.
- In some cases, aphids feed on some of the early-appearing weeds and then move to new crop plantings, thus introducing viruses which are then spread in secondary cycles within the planting.
- Bean yellow mosaic virus (BYMV) is a common problem in bean growing areas.
- Forage legumes (red clovers) are found to be the source of primary inoculum for aphids to carry BYMV into bean fields.
- For lettuce mosaic virus, only 10 to 15 seconds of feeding is needed by an aphid to acquire the virus and another 10 to 20 seconds on another plant suffices for the aphid to transmit the virus.

Exclusion

- It means preventing the entrance and establishment of pathogens in uninfected crops in a particular area.
- It can be achieved using certified seed or plants, sorting bulbs before planting, discarding any that are doubtful, possibly treating seeds, tubers or corms before they are planted and most importantly refusing obviously diseased specimens from dealers.
- In order to prevent the import and spread of plant pathogens into the country or individual states, certain federal and state laws regulate the conditions under which certain crops may be grown and distributed between states and countries.

- Such regulatory control is applied by means of quarantine, inspection of plants in the field or warehouse and occasionally by voluntary or compulsory eradication of certain host plants.
- Plant quarantines are carried out by experienced inspectors, stationed in all points of entry into the country, to stop persons or produce likely to introduce new pathogens.
- Similar quarantine regulations govern the interstate and even intrastate sale of nursery stock, tubers, bulbs, seeds and other propagative organs, especially of certain crops such as potatoes and fruit trees.
- For example, the outbreak of citrus canker in USA in 1910 through planting material imported from Southeast Asian countries.
- Due to heavy destruction, strict quarantine was imposed against entry of citrus planting material.
- However in 1981, 1984 and 1991, fresh outbreaks were reported due to illegal importation of citrus planting material. In India, interstate quarantine is in place for the movement of potato from Darjeeling area of West Bengal to prevent the spread of potato wart which is restricted to that area only.

Eradication

It involves elimination of a pathogen once it has become established on a plant or in a field. It can be accomplished by:

- Removal of diseased plants or parts as in roguing to control virus diseases or cutting off a cankered tree limb.
- Cultivating to keep down weed hosts and deep ploughing or spading to bury diseased plant debris.
- Rotation of susceptible with non-susceptible crops to starve out the pathogen.
- Disinfection usually by chemicals, sometimes by heat treatment.
- Spraying or dusting with sulphur to kill the mildew mycelium.
- Treating the soil with chloropicrin to kill nematodes and fungi.
- Soil treatment with various nematicides (Telone II, Temik 15G, Counter 15 and 20G) is useful to control sugar-beet nematodes.

Protection

- It is the use of some protective barrier between the susceptible part of the suscept or host and the pathogen.
- In most cases, a protective spray or dust applied to the plant in advance of the arrival of the fungus spores.
- Sometimes, it is achieved by killing insects or other inoculating agents.
- Sometimes it is achieved by erection of a wind-break or other mechanical barrier.
- Fungicidal sprays that act as protectants are used to control Cercospora leaf spot of sugar-beet, especially in those fields where inoculum has carried over from the previous year.
- The principle of protective fungicides is to disrupt the natural sequence of infection.
- These fungicides act on the leaf surface to kill the newly germinated spores.
- Sulphur is used as a protectant fungicide to control powdery mildew of sugar-beet.
- There is a long list of chemicals available in the literature that can be used in protective spraying and dusting, along with eradicant chemicals.
- The commercially sold chemicals are provided with instructions or notes on compatibility and possibilities of injury.
- Improvement of aeration under crop canopy reduces the humidity on aerial parts of the plant and thus checks the growth of fungi which flourish in humid atmosphere.

Immunization/Disease resistance

- Disease resistant and tolerant varieties are the cheapest, easiest and most efficient way to reduce disease losses.
- Varieties should be selected that possess resistance or tolerance to one or more disease organisms.
- For some diseases, such as the soil-borne vascular wilts and the viruses, the use of resistant varieties is the only means of ensuring control.
- Certified seed of resistant varieties is available and sold commercially.

- The use of varieties of plants resistant to particular diseases has proved to be very effective against stem rust of wheat, rust of dry bean and Rhizoctonia root rot of sugar beet.
- Most plant breeding is done for the development of varieties that produce greater yields of better quality.
- When such varieties become available, they are then tested for resistance against some of the most important pathogens present in the area where the variety is developed and where it is expected to be cultivated.
- If the variety is resistant to these pathogens for that area, it may be released to the growers for immediate production.
- There are degrees of resistance to certain diseases, some varieties being completely immune, others partially susceptible.
- Resistant varieties may become susceptible to new races of a pathogen, as happens with cereal rusts, powdery mildews, downy mildews and *P. infestans.*
- Modern DNA technology has made it possible to engineer transgenic plants that are transformed with genes for resistance against specific disease, for tolerance of adverse environmental factors or with nucleic acid sequence that lead to gene silencing of the pathogen.
- Use of microorganisms and chemicals to induce systemic acquired resistance and activations of plants' defense system could also be used for the management of plant diseases.

Therapy

- It is used on individual plants and can not be used on a large scale.
- It is achieved by inoculating or treating the plant with something that will inactivate the pathogen.
- 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 so that newly developing tissue may be obtained which is free of pathogen.

- Thermotherapy involves the exposure of diseased plants or parts of them to hot water or high air temperature for different periods of time.
- Loose smut of wheat is controlled by treating the seeds with hot water, but growing resistant varieties iss a simpler method of control.
- Hot water treatment has been used to kill nematodes in bulbs, corms, tubers and fleshy roots while they are in a dormant condition.
- Dormant chrysanthemum stools can get rid of foliar nematodes by submerging in water at 112°F (44°C) for 30 minutes.

Lecture 27

PHYSICAL AND LEGISLATIVE METHODS OF PLANT DISEASE MANAGEMENT

Aim: To acquaint the students with physical and legislative methods of plant disease management

Physical Agents Used for Disease Control

The physical agents used most commonly in controlling plant diseases are:

i) Temperature (high or low)

ii) Dry air

iii) Unfavourable light wavelengths

iv) Various types of radiations

v) Cultivation in glass or plastic green houses

vi) Plastic or net covering

Soil sterilization by heat

- Soil sterilization is completed when the temperature in the coldest part of the soil has remained for at least 30 minutes at 82°C or above, at which temperature almost all plant pathogens in the soil are killed.
- Soil can be sterilized in greenhouses, and sometimes in seed beds and cold frames, by the heat carried in live or aerated steam or hot water.
- The soil is steam sterilized either in special containers (soil sterilizers), into which steam is supplied under pressure, or on the greenhouse benches, in which case steam is piped into and is allowed to diffuse through the soil.
- At about 50°C, nematodes, some oomycetes, and other water moulds are killed, whereas most plant pathogenic fungi and bacteria along with some worms, slugs, centipedes, are usually killed at temperatures between 60 and 72°C.
- Most weeds, rest of plant pathogenic bacteria, most plant viruses in plant debris, and most insects are killed at about 82°C.
    - Heat tolerant weed seeds and some plant viruses, such as *Tobacco mosaic virus* (TMV)are killed at or near the boiling point that is between 95 and 100°C.
- Excessively high or prolonged high temperatures should be avoided during soil sterilization.

- High temperatures destroy all normal saprophytic microflora in the soil and result in release of toxic levels of some (e.g., Manganese) salts.
- High temperatures also result in the accumulation of toxic levels of ammonia (by killing the nitrifying bacteria before they kill the more heat resistant ammonifying bacteria), which may damage or kill plants planted afterward.

Soil solarization

- When clear polythene film is placed over moist soil during sunny summer days, the temperature at the top 5 cm of soil may reach as high as 52°C compared to a maximum of 37°C in unmulched soil.
- If sunny weather continues for several days or weeks, the increased soil temperature from solar heat, known as solarization inactivates (or kills) many soil borne pathogens, viz., fungi, nematodes, and bacteria near soil surface, thereby reducing the inoculum and its potential for causing disease.

Hot water treatment of propagating organs

- Hot water treatment of certain seeds, bulbs, and nursery stock is used to kill pathogens with which they are infected or which may be present in seed coats, bulbs, scales, and so on, or which may be present in external surfaces or wounds.
- Seed treatment with hot water was the only means of control in some diseases for many years, as in the loose smut of cereals, in which the fungus overwinters as mycelium inside the seed where it could not be reached by chemicals.
- Treatment of bulbs and nursery stock with hot water frees them from nematodes that may be present within them, such as *Ditylenchus dipsaci* in the bulbs of various ornamentals and *Radopholus similis* in citrus rootstocks.
- The effectiveness of this method is based on the fact that the dormant plant organs can withstand higher temperatures than those of their respective pathogens can do for a given time.
- The temperature of the hot water used and the duration of the treatment vary with the different host pathogen combinations.
- In case of loose smut of wheat, seed is kept in hot water at 50°C for 11 minutes, whereas bulbs treated for the control of *Ditylenchus dipsaci* are kept at 43°C for 3 hours.
- A short (15 seconds) treatment of melon fruit with hot (59 ± 1°C) water rinse and brushes result in a significant reduction of fruit decay while maintaining fruit quality after prolonged storage.
- Treated fruit had less soil, dust, and fungal spores at its surface while many of its natural openings in the epidermis were partially or entirely sealed.

Hot air treatment of storage organs

- Treatment of certain storage organs with warm air (curing) removes excess moisture from their surfaces and hasten the healing of wounds, thus preventing their infection by certain weak pathogens.
- Keeping sweet potato at 28 to 32^{0}C for 2 weeks helps the wounds to heal and prevents the infection of *Rhizopus* and by soft rotting bacteria.
- Hot air curing of harvested ears of corn, tobacco leaves, and so on removes most moisture from them and protects them from attack by fungal and bacterial saprophytes.
- Dry heat treatment of barley seed at 72^{o}C for 7 to 10 days eliminates the leaf streak and black chaff- causing bacterium *Xanthomonas campestris* pv. *transluscens* from the seed with negligible reduction of seed germination.

Control by eliminating certain light wavelengths

- *Alternaria, Botrytis* and *Stemphylium* are examples of plant pathogenic fungi that sporulate only when they receive light in the ultraviolet range (below 360 nm).
- Diseases can be controlled on greenhouse vegetables caused by several species of these fungi by covering or constructing the greenhouse with a special ultraviolet absorbing vinyl film that blocks the transmission of light wave lengths below 390 nm.

Drying stored grains and fruits

- All grains, legumes, and nuts carry with them a variety and number of fungi and bacteria that can cause decay of these organs in the presence of sufficient moisture.
- Such decay, however, can be avoided if seeds and nuts are harvested when properly mature and then are allowed to dry in the air or treated with heated air until the moisture content is reduced sufficiently (to about 12% moisture) before storage.
- Subsequently, they are stored under conditions of ventillation that do not allow build up of moisture to levels (about 12%) that would allow storage fungi to become activated.
- Fleshy fruits, such as peaches and strawberries, should be harvested later in the day, after dew is gone, to ensure that the fruit does not carry surface moisture with it during transit, which could result in decay of the fruit by fungi and bacteria.
- Many fruits can also be stored dry for a long time and can be kept free of disease if they are dried sufficiently before storage and if moisture is kept below a certain level during storage.
- Grapes, plums, dates and figs can be dried in the sun or through warm air treatment to produce raisins, prunes, and dried dates and figs, respectively, that are generally unaffected by bacteria and fungi as long as they are kept dry.

- Even slices of fleshy fruit such as apple, peaches, apricots can be protected from infection and decay by fungi and bacteria if they are dried sufficiently by exposure to the sun or to warm air currents.

Disease control by refrigeration

- Refrigeration is the most widely used and the most effective method of controlling post harvest diseases of fleshy plant products.
- Although low temperature at or slightly above the freezing point does not kill any of the pathogen that may be on or in the plant tissues, they do inhibit or greatly retard the growth and activities of all such pathogens, thereby reducing the spread of existing infection and the initiation of new ones.
- Most perishable fruits and vegetables should be refrigerated as soon as possible after harvest, transported in refrigerated vehicles, and kept refrigerated until used by the consumer.
- Regular refrigeration of especially succulent fruits and vegetables is sometimes preceded by quick hydrocooling or air cooling of these products, aimed at removing the excess heat carried in them from the field as quickly as possible to prevent the development of any new and latent infections.
- The magnitude of disease control through refrigeration and its value to growers and consumers is immense.

Disease control by irradiation

In this method, various electromagnetic radiations are used for controlling postharvest diseases of fruits and vegetables by killing the pathogens present on them, such as:

- UV light
- X-rays
- Gamma rays
- Particulate radiations, such as α-particles and β-particles

Legislative Methods

Quarantine regulation

Quarantine can be defined as a legal restriction on the movement of agricultural commodities for the purpose of exclusion, prevention or delay in the spread of plant pests and diseases in uninfected areas.

- Plant quarantine legislation has been placed on the statute book in most agriculturally advanced countries to restrict the movement of diseased plant material or of fungi, bacteria or viruses that can cause diseases in plants.

  Quarantine measures are of three types:

  i) domestic
  ii) internal
  iii) total embargoes

- The quarantine law was first enacted in USA in 1912, and was known as Federal Quarantine Act.
- In India, the Destructive Insect and Pest Act (DIPA) was passed in 1914 and subsequently supplemented by other provisions.
- Such quarantine laws were first enacted in France in 1660, and in Denmark in 1903.
- They aimed at the rapid destruction or eradication of barberry which has been known since early times to harbour black rust pathogen.

Some of the examples of disease which have been introduced into other countries are given below:

Disease	Year	Introduced in	Introduced from
Late blight of potato	1830	Europe	S. America
Powdery mildew of grapes	1845	England	USA
Blister rust of pine	1910	USA	Europe
Downy mildew of grapevine	1878	France	USA
Chestnut blight	1904	USA	Asia /Mediterranean region
Citrus canker	1907	USA	Asia
Bunchy top of banana	1940	USA	Sri Lanka

Examples of plant diseases introduced in India before and after the enforcement of quarantine are also below:

Disease	Year	Introduced from
Leaf rust of coffee	1879	Sri Lanka
Late blight of potato	1883	Europe

Downy mildew of grapes	1910	Europe
Downy mildew of cucurbits	1910	Sri Lanka
Paddy blast	1918	Southern Asia
Crown gall of apples and pears	1940	England
Hairy root of apple	1940	England
Bunchy top of banana	1940	Sri Lanka
Wart disease of potato	1953	Netherlands
Onion smut	1958	Europe
Golden nematode of potato	1961	Europe

Quarantine in India

- In India, there are 16 quarantine stations operating under the Directorate of Plant Protection, Quarantine and storage (DPPRS).
- Eight at sea ports, six at airports and two on land frontiers (Hussainiwala in Ferozepur in Punjab , and Sukhiapokri in Darjeeling district of West Bengal)

Destructive Insect and Pest Act

- The DIP Act in India was passed in 1914 and has been revised many times.
- A number of lacunae exit in the DIP Act; as a result, many serious plant pathogens have already been introduced in the country.
- Golden nematode (*Heterodera rostochiensis*) and wart (*Synchytrium endobioticum*) diseases of potato have been introduced in India from the European countries and are now well established in the Nilgiri Hills and Darjeeling, respectively.
- Timely action has prevented the spread of wart disease to other parts of India.
- *Synthytrium endobioticum* was first described by Ganguly and Paul in 1953 and golden nematode of potato (*Heterodera rostochiensis)* was reported by Jones for the first time in India in 1961.
- Bunchy top disease of banana was introduced into India in 1940. By 1943, it had spread to a few areas in Kottayyam and has now affected an area of about 3000 square miles in Kerala. The disease has also spread to Tamil Nadu, Orissa, West Bengal and Assam.
- In India, quarantine measures exist for two insect pests and three diseases so far.

i) Fluted scale (*Icerva purcheri*)

ii) San Jose scale (*Quadraspiditus perniciosus*)
iii) Potato wart (*Synchytrium endobioticum*)
iv) Bunchy top (virus)
v) Mosaic (virus) of banana

International Plant Protection Convention

The problem of plant diseases is global. Hence, European Plant Protection Organization (EPPO) was formed prior to the treaty in Rome. In 1951 in Rome, an International Plant Protection Convention was drawn up which at present has about 50 signatory nations. Briefly, each contracting government agrees to make provisions for:

i) An official protection organization with the specific basis of inspecting, rowing crops and the produced derived from them and issuing phytosanitary certificates.
ii) The distribution of information regarding pests and diseases both within the country and to other countries through FAO, so that a world reporting service is established.

iii) Research and investigation in the field of plant protection on a cooperative basis for diseases which have international effects. Within the framework of the international cooperation, there are six regional groups.

Lecture 28

CULTURAL METHODS OF PLANT DISEASE MANAGEMENT

Aim: To acquaint the students with cultural methods of plant disease management

Host Eradication

When a plant pathogen enters into new area despite quarantine, a plant disease epidemic may occur. All the host plants infected by pathogen may have to be removed and burnt to prevent such epidemics. This eliminates the pathogen and prevents greater losses from the spread of pathogen to additional plants.

Eradication of the crop/main host

- This type of eradication of pathogen was done in Florida and other southern states for control of bacterial canker of citrus in 1915, where more than three million trees had to be destroyed.
- Another outbreak of citrus canker occurred in Florida in 1984, and by 1992; and the disease was apparently brought under control through painful destruction of nursery and orchard trees in the United States.
- Host eradication is also carried out routinely in many nurseries, greenhouses, and fields to prevent spread of numerous diseases by eliminating infected plants that provide a ready source of inoculum within this crop.
- However, attempts to eradicate certain diseases like fire blight of apple and pear caused by the bacterium *Erwinia amylovora* and plum pox virus of stone fruits in the United States, and coffee rust in several South American countries to eradicate them have not been successful.

Eradication of the wild/volunteer host plants

- Certain pathogens of annual crops, e.g., *Cucumber mosaic virus* overwinters only or mainly in perennial wild plants.
- Eradication of host in which the pathogen overwinters is sometimes enough to eliminate completely or to reduce drastically the amount of inoculum that can cause infection in the following season.
- In some crops like potatoes, the pathogens overwinter in the infected tubers.

- These tubers produce infected plants in the spring that allow pathogen to come on aboveground parts from where it can spread further by insects, rain and wind.
- Eradication of such volunteer plants of a crop helps greatly to reduce the inoculum of these pathogens.

Eradication of alternate hosts

- Some pathogens require alternate hosts to complete their life cycle, e.g., *Puccinia graminis tritici* requires wheat and barberry, and *Cronartium ribicola* requires pine and currants.
- Eradication of wild or economically less important alternate host interrupts the life cycle of pathogen and leads to the control of the disease.

Crop Rotation

- Soil borne pathogens that infect plants of one or a few species or even families of plants can sometimes be reduced in the soil by planting non-host crops for 3 or 4 years.
- Crop rotation can reduce population of pathogens (e.g., *Verticillium*).

Fallowing

The field is tilled and left fallow for a year or part of year in some cases.

- During fallowing, pathogen debris and inoculum are destroyed by microorganism with little or no replacement.
- In areas with hot summer, fallowing allows greater heating and drying of the soil, which leads to a marked reduction of nematodes and some other pathogens.
- Other cropping systems utilize herbicides, reduced tillage and fallowing.
- In such systems, certain diseases, e.g. stalk rot of grain sorghum and corn, caused by *Fusarium moniliforme* have been reduced dramatically.
- In other diseases, such as Septoria leaf blotch of wheat and barley scab were increased.

Sanitation

Sanitation consists of all activities aimed at eliminating or reducing the amount of inoculum present in a plant, field or a warehouse and at preventing the spread of the pathogen to other healthy plants and plant products.

- Ploughing under infected plants after harvest, such as leftover infected fruit, tubers or leaves, helps cover the inoculum with soil and speed up its disintegration and concurrent destruction of most pathogens carried in or on them.
- Removing the infected leaves of house or garden plants helps remove or reduce the inoculum.
- Infected crop debris of grasses and rice crops is destroyed by burning in some parts of world, which reduces or eliminates the surface inoculum of several pathogens.
- By washing their hands before handling certain kinds of plants, such as tomatoes, workers who smoke may reduce the spread of *Tobacco mosaic virus*.
- Disinfecting the knives used to cut propagative stock, such as potato tuber and disinfecting pruning shears between trees reduce the spread of pathogen through such tools.
- Washing the soil of farm equipment before moving it from one field to another may also help in preventing the spread of pathogens present in the soil.

Practices for Creating Conditions Unfavourable to the Pathogen

- Stored product should be aerated properly to hasten the drying of their surface and inhibit germination and infection by any fungal or bacterial pathogens present on them.
- The appropriate choice of fertilizers or soil amendments may also lead to change in the soil pH, which may unfavourably influence the development of pathogen.
- In the production of many crops, particularly containerized stock, using decomposed tree bark in the planting medium has resulted in the successful control of diseases caused by several soil borne pathogens, e.g. *Phytophthora, Pythium* and *Thielaviopsis* causing root rots , *Rhizoctonia* causing damping off and crown rot, *Fusarium* causing wilt, and nematode diseases of several crops.

Polyethylene Traps and Mulches

- Many plant viruses, such as cucumber mosaic virus are brought into crops such as peppers, by airborne aphid vectors.
- When vertical, sticky, yellow polyethylene sheets are erected along edges of susceptible crop fields, a considerable number of aphids are attracted to and stick to them.
- If reflectant aluminum or black, whitish-grey or coloured polyethylene sheets are used as mulches between the plants or rows in the field, incoming aphids, thrips and possibly other insect vectors are repelled and misled away from the field.
- Reflectant mulches, however, cease to function as soon as the crop canopy covers them.

Practices for Evading or Avoidance of the Pathogen

For several plant diseases, control depends on attempts to evade pathogens.

- Bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum,* and the bacterial blight of bean caused by bacteria *Xanthomonas phaseoli* and *Pseudomonas phaseolicola* are transmitted through the seed. Therefore, they can be successfully controlled by using disease free seed and seed treatments.
- In many cases, the susceptible crop is planted at a great enough distance from field containing infected plants so that the pathogen would not infect the crop.
- Crop isolation is practiced mostly with perennial plants, such as peach orchards isolated from choke cherry shrubs or trees infected with X disease phytoplasma.
- Various activities which evade the pathogens include:

i) Using vigorous seed
ii) Selecting proper dates and proper sites
iii) Maintaining proper distances between fields and between rows and plants
iv) Planting windbreaks or trap crops
v) Planting in well drained soil
vi) Using proper insect and weed control

Such practices increase the chances that the host will remain free of pathogen or at least that it will go through its most susceptible stage before the pathogen reaches the host.

Use of Pathogen Free Seed and Propagative Material

- Seed may carry internally one or a few fungi such as those causing anthracnose and smuts, certain bacteria causing bacterial wilts, spots and blights and certain viruses (*Tobacco ring spot virus* in soybean, *Bean common mosaic virus*, *Lettuce mosaic virus*, *Barley stripe mosaic virus*, *Squash mosaic virus and Prunus nectroic ring virus*). Such diseases cn be controlled effectively by producing and using disease free seed.
- True seed, however, is invaded by relatively few pathogens, although several may contaminate its surface.
- All types of pathogen can be carried in or on propagating material.
- When a pathogen is excluded from the propagating material of the host, it is often possible to grow the host free of that pathogen for the rest of its life, e.g., woody plants, generally affected by non-vectored viruses.

Production of pathogen free vegetative propagating material

- Vegetative propagating material free of pathogens that are distributed systemically throughout the plant is obtained from mother plant that had been tested and shown to be free of particular pathogen or pathogens.
- To ensure continuous production of pathogen free buds, grafts, cuttings, rootstocks and runners of trees, vines, and other perennials; the mother plant is indexed for the particular pathogen at regular intervals.
- For certain crops, such as potato, complex certification programmmes have been evolved to produce pathogen free seed potatoes.
- For the seed to be certified the plants must show disease level no higher than those allowed by particular state.
- Sometimes it is impossible to find even a single plant of variety that is free of particular pathogen, especially of viruses. In that case, one or few healthy plants are initially obtained by meristematic tissue culture which most viruses do not invade.

Practices for the Exclusion of Pathogens from Plant Surfaces by Epidermal Coatings

- The plants are sprayed with compounds that form a continuous film or membrane on the plant surface for controlling diseases of aboveground parts of plant and inhibit contact of pathogen with the host and penetration of host.
- Water emulsion of dodecyl alcohol forms a high quality of lipid membrane. The membrane allows diffusion of oxygen and carbon dioxide but not of water. The membrane is not easily washed by rain and remains intact for about 15 days.
- Kaolin based films have also proved effective in protecting apple shoot from becoming infected by the bacterial disease fire blight, and apple fruit from powdery mildew. It also protects grapevine from Pierce disease caused by *Xylella fastidiosa* by interfering with its transmission by the vector.

Lecture 29

BIOLOGICAL METHODS OF PLANT DISEASE MANAGEMENT

Aim: To acquaint the students with biological methods of plant disease management

Biological Control- Concept

- Biological control of plant pathogens refers to the total or partial destruction of pathogen population by other organisms.
- It occurs routinely in nature. For example, several diseases in which the pathogen can not develop in certain areas either because the soil, called **suppressive soil**, contains microorganisms antagonistic to the pathogen or because the plant that is attacked by a pathogen has also been inoculated naturally with antagonistic microorganisms before or after the pathogen attack.
- Sometimes, the antagonistic microorganisms may consist of avirulent strains of the same pathogen that destroy or inhibit the development of the pathogen, as happens in **hypovirulence** and cross protection.
- Agriculturalists have increased their efforts to take advantage of such biological antagonisms and to develop strategies by which biological control can be used effectively against several plant diseases.

Suppressive Soils

- Many soil borne pathogens, such as *Fusarium oxysporum* (causing vascular wilts), *Gaeumannomyces graminis* (causing take-all of wheat), *Pythium* spp. (causing damping-off) and *Heterodera avenae* (oat cyst nematode) develop well and cause severe diseases in some soils, known as **conducive soils**, whereas they develop much less and cause much milder diseases in other soils, known as suppressive soils.
- The mechanisms by which soils are suppressive to different pathogens may involve biotic and/or abiotic factors and may vary with the pathogen.
- They operate primarily by the presence in such soils of one or several microorganisms antagonistic to the pathogen.
- Many kinds of antagonistic microorganisms have been found to increase in suppressive soils; such as *Trichoderma*, *Penicillium*, and *Sporidesmium*, or bacteria *Pseudomonas*, *Bacillus* and *Streptomyces*.

Reducing Amount of Inoculum through Antagonistic Microorganisms

a) Control of soil borne pathogens

- Several non-plant pathogenic oomycetes and fungi including some chytridiomycetes and hyphomycetes, and some pseudomonad and actinomycetous bacteria infect the resting spores of several plant pathogenic fungi.

- Among the most common mycoparasitic fungi are *Trichoderma* sp., mainly *T. viride* and *T. harzianum*.
- It parasitizes mycelia of *Rhizoctonia* and *Sclerotium* and inhibits the growth of many oomycetes such as *Pythium*, *Phythophthora*, and other fungi, e.g., *Fusarium* and *Heterobasidion* (*Fomes*).
- Other common mycoparasitic fungi are *Laetisaria arvalis* (*Corticium* sp.), a mycoparasite and antagonist of *Rhizoctonia* and *Pythium*; *Sporidesmium sclerotivorum, Gliocladium virens* and *Coniothyrium minitans*.

b) Control of aerial pathogens

- Many fungi have been shown to antagonize and inhibit numerous fungal pathogens of aerial plant parts.
- *Chaetomium globosum* and *Athelia bombacina* suppress *Venturia inaequalis* ascospore and conidia production in the fallen and growing leaves, respectively.
- *Tuberculina maxima* parasitizes the white pine blister rust fungus *Cronartium ribicola.*
- *Darluca filum* and *Verticillium lecanii* parasitize several rusts.

Control through Trap Plants

- If a few rows of rye, corn, or other tall plants are planted around a field of beans, peppers, or squash, many of the incoming aphids carrying viruses that attack the beans, peppers, and squash will stop and feed on the peripheral taller rows of rye or corn.
- Trap plants are also used against nematodes which are sedentary endo- or ecto-parasites.
- *Crotolaria* plants trap the juveniles of root- knot nematodes.

Control through Antagonistic Plants

- Plants such as asparagus and marigold are antagonistic to nematodes
- They release substances in the soil that are toxic to several plant parasitic nematodes.

Use of Resistant Varieties

- Grow varieties that have both vertical (initial inoculum- limiting) and horizontal (rate limiting) resistance and most resistant varieties have both type of resistance.
- Many of them carry only one or a few genes of vertical resistance and an unspecified number of genes of horizontal resistance.
- Such varieties are resistant only to some of the races of pathogen and if the pathogen is air borne, a new race can be brought in easily as happens with cereal rusts, powdery mildews and *Phytophthora infestans*.

- The new race virulent to the resistant variety may appear and become wide spread in this way.

Use of transgenic biocontrol microorganisms

- Genetic engineering techniques have been used to add new genes or to enhance the genetic make up of the biocontrol organisms so that it may attack the pathogen better.
- Such genes may be of plant or microbe origin that code for toxins, enzymes, and other compounds affecting the pathogen adversely, or regulatory genes that over-express appropriate biocontrol genes already present in that organism.

Direct protection by biological control agents

The most commonly used microorganisms include:

- *Gliocladium virens*, for the control of seedling diseases of ornamental and bedding plants
- *Trichoderma harzianum*, for the control of several plant pathogenic fungi
- *Trichoderma polysporum*, for the control of wood decays
- *Agrobacterium radiobacter* K-84, for the control of crown gall
- *Pseudomonas fluorescens*, against *Rhizoctonia* and *Pythium* causing damping off and other diseases
- *Bacillus subtilis*, used as a seed treatment

Biological Control of Postharvest Diseases through Fungal and Bacterial Antagonists

- Post harvest rots of several fruits could be reduced by spraying the fruits with spores of antagonistic fungi and saprophytic yeasts at different stages of fruit development, or by dipping the harvested fruit in their inoculum.
- Yeast treatments reduced post harvest rotting of peach and apple.
- Botrytis rot of strawberries was reduced by several sprays of *Trichoderma* spores on strawberry blossoms and young fruits.
- Several antagonistic yeasts protected grapes and tomatoes from *Botrytis, Penicillium*, and *Rhizoctonia* rots.

Biological Control of Postharvest Diseases through Fungal and Bacterial Antagonists

- In bacterial antagonists, *Pseudomonas* protected lemons from *Penicillium* (green mould) and pear from various storage rots.
- Two *Pseudomonas syringae* strains control the post harvest decay in citrus, apple and pear under the trade name Bio-Save.

- Stone fruits such as peaches, nectarines, apricot and plums when treated with suspensions of the antagonistic bacterium *Bacillus subtilis*, they remain free from brown rot, caused by the fungus *Monilinia fructicola* for nine days.
- *Bacillis subtilis* also protected avocado from storage rots.
- *Pseudomonas* protected lemons from *Penicillium* (green mould) and pear from various storage rots.
- Two *Pseudomonas syringae* strains control the post harvest decay in citrus, apple and pear under the trade name Bio-Save.
- Stone fruits such as peaches, nectarines, apricot and plums when treated with suspensions of the antagonistic bacterium *Bacillus subtilis* remain free from brown rot, caused by the fungus *Monilinia fructicola* up to nine days.
- *Bacillus subtilis* also protects avocado from storage rots.

Lecture 30

CHEMICAL CONTROL OF PLANT DISEASES

Aim: To acquaint the students with chemical control of plant diseases

Chemicals used for controlling insect pests, diseases and weeds are known as **pesticides**; and those used for controlling fungal diseases are called **fungicides,** those used against viruses are called **viricides**. **Antibiotics** are generally used for controlling bacterial diseases.

- One of the important or common mean of controlling the plant diseases is through chemical compounds which are toxic to the pathogens.
- These chemicals inhibit the germination, growth and multiplication of the pathogen or are lethal to the pathogen.

Classification of Chemical Pesticides

- Depending upon the pathogens they affect, they may be classified as fungicides, bactericides, nematicides, viricides etc.
- Out of these, some chemicals are broad-spectrum and they are toxic to all pathogens.
- Most of the chemicals used in plant protection are **foliar** and are used as aboveground parts of the plants.
- Some of them are soil **disinfectants**, and some are used as **protectants** for seed, tubers and culms etc. There are some of the chemicals which have been used prior to disease spread.
- A few chemicals are aimed to eradicate the general inoculum before it comes in contact with the plant hosts. They are called **eradicants** or **chemotherapeutants**.

Methods of Applications of Fungicides

Spraying and dusting

- Most important method of applying chemicals on the aerial plant parts which are exposed to different pathogens.
- Different chemicals particularly fungicides are sprayed either as protective, curative or post-symptom treatments.

- These chemicals provide a continuous covering on the vulnerable plant surfaces and do not allow the plant pathogens particularly fungi to invade them.
- They also eradicate the already established infections and reduce the secondary inoculum.
- Different equipments are available for high, low and ultra-low volume sprays in the field.
- Fungicides generally used as sprays are mancozeb, carbendazim, dodine, etc.
- Sulphur and copper fungicides can also be dusted on the crops for controlling some diseases under high humidity conditions.

Soil treatment

- Vegetables, ornamentals and trees are attacked by many pathogens which are present in the soil, like *Fusarium and Verticillium* and some bacteria.
- Different chemicals are used as soil drench, dust or granules inside the soil at the time of planting of the nursery or seedlings to control damping off, seedling blight, crown and root rot and many other soil borne diseases.
- These chemicals can also be applied with the irrigation water, wherever the irrigation is possible, especially with the drip irrigation system.
- Fungicides such as captan, metalaxyl, PCNB and chloroneb, etc. can be used as soil treatment to overcome above diseases.

Fumigation

- Most important method for controlling the nematodes and other soil borne diseases, and chemicals thus used are known as fumigants.
- Fumigants like formalin, chloropicrin, methyl-bromide, dazomet and metham sodium are now being used as fumigants in plant protection programmes.
- These chemicals are used as volatile or in gaseous form in the soil and are useful against various groups of organisms like nematodes, insects, fungi certain bacteria and weeds.

Disinfection of warehouses

- Stored products are the carrier of inoculum of many pathogens for the next season.

- These materials should be first treated with such chemicals before they are used for next planting season.
- The storage areas like rooms and the walls should also be bleached or treated with copper sulphate solution or some other sanitizing agents.

Seed treatment

- Seeds, tubers, bulbs and roots are usually treated with chemicals to prevent the pre- and post-emergence damping off of the young seedlings.
- These chemicals prevent the disease inoculum carried on the planting material.
- Since 1970's seed material is treated with the systemic fungicides to control and inactivate the pathogen in infected seed, e.g., carboxin for the control of loose smut of wheat, metalaxyl for the downy mildew of oats, etc.
- The fungicides used for seed treatment are chloroneb, captan, maneb, mancozeb, PCNB carboxin, benomyl, thiabendazole and triadimenol.
- Some are used for specific diseases and a few of them are used for controlling various type of diseases caused by fungi.
- They are applied directly on seed as dust or as thick water suspension mixed with the seed or tossed soaking with the chemical solution which is allowed to dry thereafter.

Tree wound treatment

- Fruit plants are often prone to cuts and wounds during the dormant period when they are pruned.
- The exposed portion of the plant is first sterilized by swabbing it with antiseptic solution of either sodium hypochlorite or ethyl alcohol.
- Finally, the entire wounded portion is painted with permanent tree wound dressing such as lanolin paste, Chaubattia paste or Bordeaux paste/ paint.

Post harvest treatment

- There are number of fungicides evolved for the control of post harvest diseases.

- Most of them are used as dilute solutions into which the fruits or vegetables are dipped before storage or as solution used for the washing of fruits and vegetables immediately after harvesting.
- Among the compounds used for commercial control of post harvest diseases of fruits are borax, biphenyl, sodium o-phenylphanate and widely used fungicides benomyl, thiabendazole and imazalil.

Types of chemical compounds used for plant disease control

A. Inorganic chemicals

i) Copper compounds

ii) Inorganic sulphur

iii) Carbonate compounds

iv) Phosphate and phosphonate compounds

Copper compounds

- The Bordeaux mixture (copper sulphate + calcium hydroxide), named after the region of Bordeaux of France, where it was developed against the downy mildew of grapes.
- It is still a widely used fungicide to control many diseases like bacterial leaf spot, blights, anthracnose, downy mildews and cankers throughout the world.
- Phytotoxicity of Bordeaux mixture can be reduced by increasing the ratio of hydrated lime to the copper sulphate.
- Copper oxychloride (Brand names: Blitox 50, Blue copper, Fytolan, etc.) is used to control diseases caused by oomycetes and cankers of fruit trees.

Inorganic sulphur

- Elemental sulphur is known as the oldest fungicide.
- It is used as a dust, wettable powder, paste or liquid formulation.
- It primarily controls powdery mildews, certain rusts, leaf blights and fruit rots.
- These are available in different trade names like Sulfex, Wettasul, Cosavet etc.

Carbonate compounds

- Sodium bicarbonate, as well as bicarbonate salts of ammonium, potassium and lithium are used as fungicides.
- These compounds plus 1 per cent superfine oil are inhibitory and fungicidal to the powdery mildew fungi on roses, grey mould and southern blight fungus.

Phosphate and phosphonate compounds

- Spraying cucurbits or grapevines with either of monopotassium or dipotassium phosphate gives satisfactory control of powdery mildew diseases of these two hosts.

B. Organic Chemicals

i) Organic sulphur compounds or dithiocarbamates

ii) Quinones

iii) Aromatic compounds

iv) Heterocyclic nitrogenous compounds

Organic sulphur compounds or dithiocarbamates

- Organic sulphur compounds form the most versatile and widely used group of modern fungicides.
- This group includes thiram, ziram, ferbam, nabam, maneb, mancozeb and zineb.
- They are the derivatives of dithiocarbamic acid which are toxic to fungi due to isothiocynate radicals and inactivate the sulphydral (SH) group in amino acids and enzymes within the fungus cells.

Quinones

- Quinones occur naturally in many plants and are used as fungicides.
- Only two quinone compounds chloranil and dichlone are used.

Aromatic compounds

- Many unrelated compounds that have benzene ring in centre are toxic to microorganisms, and several of them have been used as fungicide.
- Penta-chloro-nitro benzene (PCNB) sold as Brassicol is a long lasting soil fungicide which controls various soil borne diseases of vegetables and ornamentals and is applied as dip or furrow treatment.
- Another fungicide dichloran (DCNA) sold as Botran is widely used against diseases caused by *Botrytis*, *Sclerotinia* and *Rhizopus.*
- Chlorothalonil available as Bravo, Daconil and many other brand names is excellent broad-spectrum fungicide and is used against many leaf spots, blights, downy mildews, rusts, anthracnose, scab and fruit rots of fruits and vegetables.
- Biphenyl is used against various diseases caused by *Penicillium, Diplodia, Botrytis* and *Phomopsis* in case of citrus.

Heterocyclic nitrogenous compounds

- This group includes important fungicides, like captan, captafol and folpet.
- Captan is excellent fungicide for control of leaf spots, blights and rots of many fruits and vegetables and is used as seed treatment and foliar spray.

Systemic Fungicides

Acylalanines

- Most important fungicide in this group is metalaxyl which is effective against oomycetes, like *Pythium, Phytophthora* and downy mildews.

Benzimidazoles

- They include some of the systemic fungicides like benomyl, carbendazim, thiabandazole and thiophanate methyl.
- Benomyl (which is sold as Benlate) and carbendazim (sold as brand names, like Bavistin, etc.) control various types of diseases like leaf spots, blights, rots, scab and seed borne diseases; but are not effective against oomycetes and dark colour spore forming fungi including *Alternaria* spp.

- They are effective against powdery mildews on many crops, apple scab and brown rot of stone fruits.
- Thiophanate methyl sold as Topsin M, is a broad-spectrum fungicide is also used against powdery mildew of various crops.

Oxanthiins

- They were the first to be discovered as having systemic fungicide activity.
- Carboxin is sold as Vitavax used against damping-off disease caused by *Rhizoctonia* and various smuts of grain crops.
- Oxycarboxin- marketed as Plantvax is effective against wheat rusts.

Organophosphates

- They include primarily fosetyl-Al, sold as Aliette which is very effective against many foliar, root and stem diseases caused by oomycetes such as *Pythium*, *Phytophthora*, and downy mildews in a variety of crops.
- Fosetyl-Al has been reported to stimulate defence reactions and the synthesis of phytoalexins against oomycetes.

Pyrimidines

- They include dimethirimol (Milcurb), ethirimol (Milstem) and bupirimate (Nimrod), all of which are effective against powdery mildew of various crops.
- Fenarimol (Rubigan) and Nuarimol (Trimidal) are effective against powdery mildew and also other scab, leaf spot, rust and smut diseases.

Triazoles

- Triazoles (-conazoles or imidazoles) include several excellent systemic fungicides such as triadimefon, bitertanol, difenoconazole, propiconazole, myclobutanil, cyprconazole and tebuconazole, etc.
- They show long protective and curative activity against broad-spectrum of foliar, root and seedling diseases like leaf spot, blights, powdery mildew and rusts causing fungi.

- They can be applied as foliar as well as seed and soil treatments.

Strobilurins

- These are the latest fungicides, also known as QoI fungicides.
- The most important strobilurin fungicides are azoxystrobin, trifloxystrobin and kresoxim methyl.
- These strobilurins can be used for controlling the diseases of grapevines, pome and stone fruits, cucurbits, sugar beet and rice.

Lecture 31

USE OF RESISTANT VARIETIES IN PLANT DISEASE MANAGEMENT

Aim: To acquaint the students with use of resistant varieties in plant disease management

Introduction

- Use of resistant varieties for crop cultivation provides the most cost-effective, the easiest, and the safest of all the methods used for disease control.
- Both from economic point of view, and the possible health hazards involved in some of other methods of disease control, this can probably be termed as the **"painless method"**. This approach costs little to the farmer and is, therefore, suitable for the developing countries like India.
- Cultivation of resistant varieties provides probably the only means of producing acceptable yields without using toxic compounds for many diseases like the vascular wilts, viral diseases, cereal rusts, powdery mildews, and root rots, etc.
- Several other kinds of fungal diseases and also many others caused by bacteria, nematodes, and viruses are best controlled by this approach.
- However, resistant varieties could be effectively used only in limited number of cases against the diseases of forest and fruit trees, e.g., blister rust of white pine (*Cronartium ribicola*), fusiform rust of pine (*C. quercuum* f. sp. *fusiforme*), and apple scab (*Venturia inaequalis*).
- It is always preferable to use resistant host varieties that have both **vertical and horizontal resistance**.
- Most resistant varieties have only one or few (2 - 3) genes for vertical resistance (mono- or oligogenic resistance, respectively) and an unspecified number of genes for horizontal resistance (polygenic resistance).
- Where inoculum production is rapid and its buildup high, and it is air borne, new races of the pathogen may appear quite often and soon become widespread. Such examples are cereal rusts, powdery and downy mildews and late blight of potato.

Resistance break down

- As the new race takes over, resistance of the old variety is no longer effective.
- Depending on the genetic plasticity of the pathogen and the particular gene or combination of genes involved in host resistance, resistant varieties with only vertical resistance, need to be replaced periodically.

- This means that breeding programmes for new resistant varieties has to continue so that some new varieties can be kept in readiness for the replacement of the old ones in case of any eventuality.
- It is hoped that genetic engineering techniques would come to the aid of such breeding programmes and make it possible for a quick transfer of individual genes or a combination of such genes to preferred susceptible host varieties in a much shorter time.

Maintenance of resistance

- Disease management strategies, such as sanitation, seed treatment or use of fungicide reduce the exposure of resistant variety to large pathogen population.
- For pathogens with low inoculum production and slow dispersal rate, resistance of the host variety usually lasts longer.
- The use of varietal mixtures has been widely used in a variety of crops as a possible measure in disease control in cereals, legumes and potatoes.
- A cultivar mixture is simply compounded by mixing seeds of cultivars on the basis of their predicted performance.
- Diversification of resistance naturally presents the pathogen with a difficult target than in the traditional monoculture.

Multiline varieties

- Jenson (1952) first proposed the idea of **multiline varieties** that is a composite of various isogenic lines sharing most agronomic characters, but carrying different genes for vertical resistance in one or a few of its constituents of the multiline variety.
- Use of multiline variety results in overall reduction of pathogen for a disease, which consequently reduces the rate of disease and also the inoculum presence on each of the component varieties.
- The most fully developed multiline programme involved wheat rusts and crown rust of oats.
- Multilines can delay the onset of disease and also reduce the rate of an epidemic.
- If a constituent variety loses its resistance to a new race of the pathogen, it can be replaced by a suitable alternative line.
- There are, however, certain limitations on the use of multiline varieties.
- The components must be distinct from each other, have different race-specific genes, and also ripen simultaneously.

Reliable resistance

- It is now accepted that crop resistance based on single or few vertical resistant genes is liable to become nonfunctional soon, mostly within 4 years.
- In the long run, the production of varieties with many additional genes for horizontal resistance may perhaps provide the only answer.

Breeding of resistant varieties

- Quite early in the twentieth century it became evident that breeding of resistant plant varieties was possible, and this provided the most desirable approach to plant disease control.
- The environment pollution in chemical control further highlighted the importance of such breeding.
- Plant breeding represents the most significant form of biological control of plant diseases.
- Genetic diversity can be regularly introduced into the plant genome through such breeding programme.
- Cultivated crop plants that we see today represent the results of natural selection or selection and breeding of different lines that evolved naturally in different regions over many thousands of years.
- It has been a very slow process.
- Many of them still exist as wild types at the place of their origin and have survived over such long periods in attack of various pathogens, because of many resistance genes they carried and also gradually acquired through natural crossing within the plant population.
- Weak and susceptible ones were eliminated in course of time.
- The survivors had sets of major and minor genes for resistance and much genetic diversity, adapted to the local health environment and suited to the needs of local population.
- Numerous varieties of each crop plant are cultivated throughout the world and they represent a non-uniform population.
- Widespread systematic efforts of plant breeders all over the world have further increased this diversity.
- Now, biotechnology has come in a big way with techniques aimed at further increasing this.
- The first step in breeding for disease resistance is mostly to decide on type and level of resistance required and whether the pathogen is seed-, soil- or air-borne.

- The decision will depend on the availability of a suitable source of resistance and whether or not it can be manipulated in a breeding programme.
- Many plant diseases cannot yet be properly controlled by host resistance, for example, powdery mildews of cereals, as this is complicated by pathogenic specialization and a complex resistance pattern.

Source of resistance

- Search for resistance is initially restricted to crop cultivars currently in use locally.
- Search has to be widened to include varieties grown in the adjacent regions, wild plant relatives, and species growing in the area where the disease is severe, or where the disease is originated.
- Plant breeders often take recourse to creation of new resistant genotypes for this purpose by inducing mutation or approach gene banks maintained in different countries.
- Larger public collections are maintained in different countries.

  -United States Department of Agriculture (USDA) for many crop species at Beltsville, Maryland, USA

  -CIMMYT at Londres, Mexico, for maize and wheat

  -International Rice Research Institute (IRRI) at Los Bagos, Phillippines, for rice

  -International Crop Research Institute for Semi Arid Tropics (ICRISAT) at Hyderabad, India, for legumes and small grain cereals.

Methods of developing resistance

There are three common methods of developing resistance in the host.

i) Selection ii) Hybridization iii) Mutation

- **Selection** is an old practice of developing resistant varieties. When a large number of individuals grow under disease favourable environment, some individuals show some resistance to the disease which might be selected and tested again before recommendation as a resistant variety.
- **Hybridization** involves the crossing of two individuals (parents) with good commercial qualities lacking resistance to specific pathogens and another, a source of resistance lacking desired commercial traits.

- The source of resistance can be obtained by selection from variety or species much prevalent in the area.

- If such variety is not available in the area under cultivation in cultivated varieties or species, the desired individual can be obtained from some other species or related wild plants.

- Successful crossing of wild *Lycopersicon pimpinellifolium* with cultivated tomato *Lycoperiscon esculentum* has produced material for the development of varieties resistance to Fusarium wilt.
- The varieties developed by hybridization and selection for disease resistance in different vegetables are: Tomato (Hisar Anmol, Hisar Gaurav, H-86 and H-88 against *Tomato leaf curl virus*), brinjal (Pant Rituraj against bacterial wilt), pea (Palam Priya, JP 83 against powdery mildew), cowpea (Pusa Komal against bacterial blight), cauliflower (Pusa Shubhra against black rot and curd and inflorescence blight), cabbage (Pusa Mukta against black rot, Pusa Drumhead against black leg), watermelon (Arka Manik against anthracnose, powdery mildew and downy mildew), etc.

- **Mutation** is a sudden heritable change in the genetic makeup of the individual plant. In nature, chance mutations are possible, however, little success has been found for developing resistant varieties by this method in the field. The variety Pusa Parvati of French bean resistant to mosaic and powdery mildew diseases; and variety Punjab 8 (EMS 8) carrying field resistance to yellow vein mosaic have been developed by this method.

- Newly developed resistant plants have to be tested for resistance after artificial inoculation with the pathogen or natural infection under field conditions.

- Recently, molecular markers have been used in place of such inoculation for the selection of resistance, at least in the early stage of breeding.

- Resistance is not always stable and may also fail to function under certain conditions.

- To minimize such possibilities, precise standards have been set in respect of conditions for inoculation, environmental conditions in which inoculated plants are to be kept, and assessment of disease symptoms and incidence.

While searching for resistant genotypes, selection is done from existing crops in the following way:

i) Mass selection

ii) Pure line selection

iii) Pedigree selection

iv) Bulk hybrid method

v) Recurrent selection

vi) Other techniques

Mass selection

- Seeds are collected in mass from some selected, highly resistant plants surviving in a cropped field where natural infection occurs regularly, and seeds are composited after harvest for use in the next season.
- This method is no doubt simple, but plant improvement is slow. Further, in cross-pollinated plants there is no control over the source of pollen.
- In onion, the variety Arka Kalyan is resistant to purple blotch and is developed by mass selection only.

Pure line selection

- In pure line selection, seeds are collected only from individual highly resistant plants, and the progenies are grown separately.
- They are repeatedly inoculated with the target pathogen for disease resistance.
- This method is very effective for self-pollinated crops but not so with cross-pollinated ones.
- No new genotype is created by this method, which simply isolates the best genotype present in a mixed population.
- This, however, represents a more rapid method than allowing natural selection to take place and eliminates the more susceptible genotypes.
- Traditionally, mass or pure line selection methods are adopted for heterogenous plant populations. In chilli, the varieties G 4 is fairly tolerant to diseases, NP 46A is tolerant to virus, Arka Lohit is tolerant to powdery mildew and Musalwadi Selection is tolerant to powdery mildew and dieback diseases. These varieties were developed by pure line selection method.

Two more procedures of selection are commonly followed after hybridization to sort out desirable genotypes from the segregating progeny. These are **pedigree selection** and **bulk hybrid** methods.

Pedigree selection

- In this method of selection, plants with desired combination of characters are selected in the F_2 generation after hybridization between two homozygous lines carrying different genes for resistance.
- Their progenies are propagated separately and inoculated, and the progenies of each selected plants are maintained in succeeding generations for resistance.
- These steps are continued up to F_7 or F_8 generation, when a high degree of homozygosity is achieved. This method takes advantage of the phenomenon of **heterosis** (hybrid vigour).
- The disease resistant varieties developed by pedigree selection in different vegetables are: Chilli (Pant C1 tolerant to chilli viruses; Punjab Lal resistant to TMV, CMV and leaf curl viruses; Jawahar 218 tolerant to leaf curl and fruit rot; Pusa Sadabahar, Jwalamukhi and Jwala Sakshi resistant to viruses), Okra (Parbhani Kranti, Arka Abhay, Arka Anamika and Punjab Padmini highly tolerant to YVMV), etc..

Bulk hybrid method

- This method is practiced following hybridization between two selected parents.
- Their seeds are bulked, grown out again, and the process is repeated.
- At each generation, plants are exposed to natural infection or artificial inoculation with the pathogen and reselected for resistance.

Recurrent selection

When it is desired to quickly introduce a single simply inherited, dominant, resistant character into an existing susceptible plant with desirable agronomic qualities, a back-cross or recurrent selection is adopted. This involves a succession of crossing of the 'donor' plants with the dominant resistant progeny with the existing cultivar, i.e. the recurrent parent, ultimately consolidates the resistant gene in the genetic background of the desirable susceptible variety. However, this method is time-consuming, and not equally effective in all cases, particularly for the self-pollinated plants.

Other techniques

Some other techniques are also occasionally used for introducing disease resistance in plants.

- Both natural and artificially induced mutants that exhibit improved resistance and a change in the chromosome number in plants or production of euploids (4N, 6N) or aneuploids (2N + 1 or 2 chromosomes) by the use of mutagenic chemicals like colchicine have also shown good effect in some cases.

Lecture 32

INTEGRATED PLANT DISEASE MANAGEMENT

Aim: To acquaint the students with Integrated Plant Disease Management

Integrated Disease Management

Integrated disease management (IDM) came under focus in 1960's when chemicals especially, fungicides and insecticides came under the attack from environmentalists due to the overuse of chemicals that created the problems of environmental pollution, chemical residues in food stuff, land, water and air, and the associated health hazards.

- It focused on the other methods of disease control.
- It involved cultural, biological, epidemiological and alternative means to achieve the disease control.
- Nowadays, there is an emphasis on disease "management" rather than on "Control".

Definition of IDM

"**Disease management system that in the context of associated environment and population dynamics of microorganisms, utilizes all suitable techniques and methods in a manner as compatible as possible and maintains the disease below economic level"**.

- In general, it is the integration of all possible and suitable management techniques for the control of diseases.
- The practices which need to be avoided in IDM are indiscriminate use of fungicides, monoculture and growing of susceptible cultivars.
- Integrated disease management ensures the proper management of soil health, use of healthy seeds and planting material, application of fungicides when required, field sanitation, cultural practices which suppress the disease , use of bio-control agents and growing resistant plant genotypes .

Different Approaches of Integrated Disease Management System

1. **The combined control approach**

   It is a combination of control methods like adjustment in sowing time, seed treatment, use of resistant variety, chemical spray schedule etc. This type of IDM is widely practiced as a package of practice where the occurrence of disease is certain and sure.

2. The surveillance based approach:

It is an advanced IDM approach based on crop health monitoring and surveillance, and takes into account the economic threshold levels or economic damage levels.

3. Advanced integrated disease management system

It involves the high input technology like computer supported forecasting, remote sensing, scouting, multiple pathogen thresholds, information on life cycle of pathogens, epidemiology of diseases, environmental factor and knowledge based decision making.

Main components of integrated disease management (IDM)

1. Host resistance
2. Induced systemic resistance
3. Genetically improved plants
4. Cultural practices
5. Physical methods
6. Plant nutrition
7. Biological control
8. Use of pesticides of plant origin
9. Judicious use of chemicals

Host resistance

- Resistant varieties can be the simple, practical, effective and economical method of plant disease control.
- Apart from ensuring protection from diseases, they can also save time, money and energy spent on other methods of control and avoid environmental pollution with chemicals.
- They are the only practical method of controlling such diseases as wilts, rusts and others caused by viruses in which chemical control is very expensive and impractical.
- In low value crops, where other methods are often too expensive, development of varieties resistant to common and important diseases can be an acceptable recommendation for the farmers.
- Disease resistance in plants is also governed by their genetic constitution and can be monogenic, oligogenic or polygenic.

Advantages of host plant resistance

- No adverse effect on environment and man, rather the resistant cultivars put a constant and cumulative effect on pathogen.
- Host plant involves no extra cost to the farmers and does not require inputs and application skills.

Disadvantages of host plant resistance

- The development of pathogen resistant variety takes 5-10 years.
- Host plant resistance can put a selection pressure on pathogen to the extent that it may lead to the evolution of new biotypes of pathogen.

- Introduction of varieties with resistance to one pathogen leads to the emergence of new pathogen problem because of the absence of competition from the key pathogen.

Induction of host resistance

- Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability.
- Plants also respond to a variety of chemical stimuli produced by soil- and plant-associated microbes.
- Such stimuli can either induce or condition plant host defence through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens.
- Induction of host defence can be local and/or systemic in nature depending on the type, source, and amount of stimuli.
- The **systemic acquired resistance** (SAR) is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins.
- These PR proteins include a variety of enzymes, some of which may act directly to lyse the invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death.
- Whereas, the **induced systemic resistance** (ISR) is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some non-pathogenic rhizobacteria.
- Interestingly, the SA- and JA- dependent defense pathways can be mutually antagonistic, and some bacterial pathogens take advantage of this to overcome the SAR.
- Pathogenic strains of *Pseudomonas syringae* produce coronatine, which is similar to JA, to overcome the SA-mediated pathway.
- Because various host-resistance pathways can be activated to varying degrees by different microbes and insect feeding, it is plausible that multiple stimuli are constantly being received and processed by the plant.
- Thus, the magnitude and duration of host defence induction will likely vary over time.

Genetically improved plants

- Genes from plants, microbes and animals can be combined and introduced in to the living cells of other organisms, and the organisms that have genes from other species inserted into their genome are called **transgenics**.

- Production of disease resistant transgenic plants has been achieved by this method; certain genes are inserted in to plant genome that confer resistance to pathogens such as viruses, fungi and insects.
- These transgenic plants reduce the pesticide use and thereby provide environmental benefits while reducing farmers cost.
- Genetically modified plants are generally used to control the viral diseases, e.g., a transgenic papaya cultivar 'Rainbow' has been developed which is resistant to *papaya ring spot virus* in the US.

Integration of different cultural practices

- Different cultural practices like crop rotation, mulching, tillage, different soil amendments, soil solarization, soil sterilization, change in date of sowing, plant spacing etc. when applied alone are able to control diseases up to some extent; but when these cultural practices are combined with each other, they not only control the diseases but also increase the yield of crops.
- The inter-cropping of maize and sorghum with peppers serves as barriers against the aphid vectors of pepper veinal mottle virus and reduces the virus spread.
- Soil solarization for 40 days along with the addition of cabbage, cauliflower, broccoli and sarson leaf residues controlled the gladiolus wilt (*Fusarium oxysporum* f.sp. *gladioli*) by 74.6% whereas soil solarization (for 40 days) alone reduced the gladiolus wilt by 67.3% compared to the un-solarized control.

Physical methods of disease control

- Solar heat treatment of the water soaked wheat seed in May-June for 5-6 hours provides good control of loose smut of wheat.
- Most of the post harvest diseases can be avoided by irradiation, refrigeration, Controlled Atmosphere Storage etc.
- Soil solarization has been used to control soil borne diseaseS caused by otherwise difficult to control fungi, e.g., *Rhizoctonia solani, Fusarium* spp., *Sclerotium* etc .
- In this the soil beds are first irrigated and then covered with thin (20 µm) transparent mulch in the months of April, May and June.
- It raised the soil temperatures in some cases up to 50^0C, which is deleterious to many plant pathogens in the soil.
- It has been used in raising disease free nursery in tropical and subtropical climatic areas. It also provides excellent weed control.
- Hot water treatment of cabbage seed at 52^0C for 15-20 minutes controls black rot disease (caused by *Xanthomonas campestris* pv. *campestris*).

Plant nutrition

- The nutrition of crop plants has direct effect on the diseases, and is an important component of integrated disease management (IDM).
- Both deficient and over-nourished plants invite high incidence of diseases as well as loss in yield and quality of produce and products.
- The amount, proportion, time and method of application of fertilizers affect the metabolism of plants and thus occurrence and severity of diseases.
- Fertilization with both P and K significantly reduces the leaf rust damage and powdery mildew infection in wheat.
- The deficiency of macronutrients may also affect the incidence of many diseases.
- Potassium (K) plays an important role in survival of crop plants under environmental stress conditions.
- Potassium also affects the reaction of plants to pests or diseases by having direct effect on the pathogen number, development, multiplication, survival, vigour and length of life cycle.

Biological control

- Biocontrol agents are used as a core component of integrated disease management system.
- The science and art of using living organisms as biocontrol agents is an important component of environment friendly disease management procedures.
- These biocontrol agents are of enormous value in integrated diseases management for sustainable agriculture where they often replace the need of fungicides.
- The biocontrol agents either suppress the pathogen growth either by the antibiotic production, hyperparasitism or by competition.
- Various biocontrol agents used in control of various diseases are *Bacillus subtilis, Pseudomonas fluorescens, Gliocladium* spp., *Trichoderma* spp., *Chaetomium globosum, Pseudomonas cepacia, Bacillus cereus, Agrobacterium radiobacter* etc.
- *Trichoderma viride* is the most important and versatile biocontrol agent used for the control of a number of plant pathogens like *Rhizoctonia solani* and *Sclerotium rolfsii* which are otherwise difficult to control by other methods.
- Similarly, *Fusarium lateriticum* has been used to cover primary wounds of apricot for avoiding the canker disease caused by *Eutypa armeniacae.*

- Application of *Peniophora gigantea oidia* paste on pine stumps provided effective control of *Heterobasidion annosus* root rot disease which spreads through unprotected stumps left over after felling.
- *Ampelomyces quisqualis* and *Darluca* spp. hyperparasitize powdery mildew and rust fungi, respectively, and therefore exploited for their biological control.
- *Agrobacterium radiobacter* K-84 strain has been used against crown gall disease world over.

Use of pesticides of plant origin

- Pesticides of plant origin are derived from plant parts and their genes are also used to transform crops to express resistance to insect, fungal and viral attack.
- The plant parts and their extracts with antifungal properties play an important role in plant disease management.
- Plants with pest killing properties have been recorded as early as Rig Veda in India.
- Garlic (*Allium sativum*) has a long history of reputed value and actual use for its medicinal, antimicrobial and pesticidal properties.
- The growth of *Rhizoctonia solani* can be reduced with ethanolic extracts of *Eucalyptus* sp., *Chenopodium ambrosioides*, *Lippia alba*, *Aegle marmelos* and *Cestrum diurnum* leaves.
- The seed extract of *Piper nigrum* was found to be effective against *R. bataticola*.

Judicious use of fungicides

- Chemicals have been used successfully to combat the ravages of these diseases for many years.
- Fungicides with different modes of action like protective (broad spectrum fungicides), post infection activity (EBI), pre- symptom and post symptom (benzimidazoles and triazoles) may be used for controlling a wide array of plant diseases ravaging various crops.
- The over-use of these chemicals resulted in water pollution, residues on food and fruit crops, effect on non- target organisms and development of resistance in pathogens against the chemicals have drawn the attention toward the rational use of fungicides by including monitored control strategies and cultural practices.

Types of Integrated Disease Management

i) Integration of cultural and chemical control

- The integration of chemicals and cultural practices (including improved cultivars) has resulted in a continuous supply of fresh watermelons, reduced diseases caused by

Colletotrichum lagenarium, *Pseudomonas syringae* pv. *lachrymans* and *Pseudoperonospora cubensis*.

- The covering the tomato nursery seedlings with nylon net for 25-30 days plus 4 sprays of monocrotophos at 10-days intervals after transplanting, delayed the spread of *Tomato leaf curl virus* for 3-5 weeks and increased tomato yields.

ii) Integration of chemical and biological control

Bio-control agents such as *Pseudomonas fluorescens, Trichoderma viride, T. harzianum, Bacillus subtilis, Pseudomonas putida, P. cepacia, Talaromyces flavus,* and *Agrobacterium radiobacter* strain K 84 etc. can be used with integration of chemicals for the effective control of certain diseases.

iii) Integration of resistance, cultural, biological and chemical control

The integration of cultural practices (crop rotation, good farm hygiene procedures, quarantine), fertilizers, soil fumigation and solarization, pesticides (fungicide transplant dips, soil drench, soil incorporations, seed treatments, trace elements and surfactants), resistant cultivars and biocontrol agents are used for the control of club root (*Plasmodiophora brassicae*) of vegetables.


**********Thanks**********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