Detection, Diagnosis and Management of Plant Diseases

Detection, Diagnosis and Management of Plant Diseases

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BURNABY, BRITISH COLUMBIA, CANADA

Contents

Course Description

Management of crop diseases caused by various phytopathogens needs proper detection and diagnosis of the causal agents. Adequate crop management strategies could be sorted out only when the actual causal agent is correctly established. The course is designed to discuss the approaches used for plant disease detection and diagnosis. Both conventional, as well as advanced molecular diagnostic techniques currently being used for plant disease diagnosis, will be discussed. Additional emphasis will also be given to discuss the recent advancements in plant disease diagnostics and special applications of diagnostic tools for the diagnosis of specific plant pathogens. Additionally, how plant disease diagnostics can help in the management of plant diseases will also be discussed. In short, the course is designed to present a clear picture of the concepts of disease detection and diagnosis; tools and techniques used for disease detection and diagnosis; special applications of plant disease diagnostic tools; diagnostic challenges; forensics of plants and microbes; and diagnostics in plant disease management.

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Course Content

- Detection of plant pathogens
- Conventional diagnostic techniques
- Advanced and Molecular diagnostic techniques
- Recent advances in diagnostic techniques
- Special applications of plant disease diagnosis
- Diagnostics in plant disease management

Course Audience

- UG and PG students of Agriculture and Allied Sciences
- Faculty of SAUs
- Agriculture Scientists in ICAR
- Professionals in the State Department of Agriculture
- Specialists working in KVKs
- NGOs in Agriculture
- Progressive farmers/ Farming community

Outcomes of this Course

- Biotechnology & Genomics in plant disease management
- Procedures in plant disease diagnosis
- Identification and quantification of airborne inoculum
- Diagnosis of seed-borne pathogens
- Genomics & AI-based diagnosis
- Detection of human pathogens on plants
- DNA barcoding of pathogens of quarantine

PART I

WEEK 1: DETECTION OF PLANT PATHOGENS

1 Detection vs. diagnosis – Definition and Differences

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Transcript

Hello everybody!! I welcome to the course Detection, Diagnosis and Plant Disease management. The first talk in the discourse will be dealing with detection versus diagnosis there are certain differences between these two terms and we'll try to understand the differences of these two terms we all know that healthy plans are required to feed the world but unfortunately plant suffers from varieties of plant pathogens and that is why course is necessary to go for detection and diagnosis of plant pathogens healthy crops are essential for safe healthy and sustainable farming that contribute to the quality of food and life reliable Diagnostics for the timely detection of plant pests and diseases provide the basis for healthy crop production this is how Diagnostics help controlling risk and provide security during crop production now let us try to understand what is a plant disease any disturbance of a plan that interferes with its normal growth and development can be called as plant disease plant disease can be caused by either by at occasions or a biotic causes and plant diseases caused major production and economic losses in agricultural industries globally early information

on crop health and disease detection can facilitate the control of diseases as well as minimize economic losses that is why it is highly important to detect and diagnose the causal result of disease and that is so that we can facilitate that control of the disease and thereby minimize loss to the growers how detection is different from diagnosis in practice to detect a problem is to objectively observed symptoms caused by the problem whereas to diagnose a problem is to ascertain the specific pathological condition that is causing the problem so this is the basic difference between the detection and diagnosis identification of disease-causing agent may take a week or more time the following expects are important during identification that is to use the power of observation and ask questions related to the disease in order to eliminate or identify possible causes of the problem and also to consider various environmental and cultural factors that are associated with the plants that are suffering from a particular disease as a result of the questions and of the resistance of the diagnostician he or she may be able to identify a disease and this is causingasn't or he be able to narrow the problem down to several possibilities which will require further study in the laboratory or even the diagnose station may be completely baffled by the problem it may be if it is a new problem or an emerging problem so to diagnose or detect before going for detection and diagnosis of the plant disease it is absolutely important to know what is normal for that particular plant for example in case of certain plant the proper identification of the plant type is highly important secondly recognizing healthy appearance of the plant is also to be in the back of the mind for the diagnostician to distinguish it from a normal and diseased plant for example some of the plants they have some genetically variation in the manifestations of their leaf color or in the flower color or in the leaf structure and these manifestations this is not considered to be abnormal as these manifestations are inherent characteristics of the plant itself for example yellow coloration in the leaf in many plants then leaf distortion in the colocasia plants and color breaking in tulip flowers so these are normal 4d plant species and it should

not be considered as a disease plant so identification characteristic symptoms for plant diseases are basically based on under development of tissues or organs over development of tissues or organs necrosis or death of plant parts or alteration of normal appearance all these are characteristic symptoms of disease then again there is a two term sign and symptoms and these symptoms are basically changes in the plant's appearance in response to a pathogen that deviates from its normal appearance for example common bacterial blight symptoms include brown necrotic lesions surrounded by a bright yellow halo at the leaf margin or interior of the leaf on bean plants at the same time dizzy signs are the structures of the plant pathogen visible on the infected plants for example fruiting bodies of fungus such as powdery mildew on leaf is the parasitic fungal disease organism itself so this is the basic distinction between sign and symptoms as where the sign manifestation of sign is the presence of structures of the pathogen whereas symptoms is the change or deviation from the normal appearance of the plant or plant part the fungal disease science includes say for example leaf rust stem rust in sclera tinea white mole or in powdery mildew in all these diseases one can have manifestation of the presence of the fungal structures for example on leaf rust these early pustules of the rust pathogens then in case of squirting near white mold we can see the presence of fungal on the beans then fungal diseases symptoms includes bird's eye symptom on berries wilting of plants leaf spot chloro sill closes in all these cases we see the just the symptoms on the leaf or other plant part but not the Paterson itself or Paterson structures itself so this is the basic difference between science and symptoms and here are some of the manifestations of fungal diseases signs and symptoms similarly there are signs and symptoms also present for bacterial disease normally Bechtol disease signs are difficult to observe but it can include bacterial coos water-soaked lesions bacterial streaming from cut stem and so on since bacteria are very smaller in size one cannot see bacterial cells with normal eyes and that is why some characteristic symptoms like bacterial poos water-soaked legions

bechtel streaming can be considered as signs of bacterial disease whereas typical bacterial symptoms include leaf spot with yellow hello fruit spot canker crown gall all these part of bacterial disease symptoms similarly for viral disease since virus also we cannot see with our naked eye so there won't be any signs of viral structures or viruses that will be able to make it out with our naked eyes but definitely viral disease symptoms can be very well identified based on the mosaic type of symptoms crinkle leaves yellowed leaves plant stunting these are some of the characteristic symptoms of viral disease at the same time a plant may be affected by more than one viruses and it can cause variation in the symptom production in the same plant species so one has to have thorough understanding of these signs and symptoms that are produced by different plant pathogens on a particular plant species to correctly identify the pathogen at the very beginning say for example in peach shielding when it is infected by three different combination of viruses of the same plant species they produces different symptoms on the same host for example if the left one implant is of Beach is infected by prune dwarf virus as well as pruners necrotic ringspot virus whereas the prune dwarf virus infection is in the middle and in the right hand plant it is infected by pruners necrotic ringspot virus so different viruses can have different symptoms produced on the same host and if this combination of more than one virus infection then it may lead to again production of entirely different type of symptoms so all this has to be kept in mind for the diagnostician or the plant pathologist who is at the investigating level to determine the signs and symptoms along with the cause possible causal agent that may be associated wind up the pathogen a host and with this we have come to an end of the first talk of the week and till we meet in the second talk have a good time.

Thank You

PDF: Detection vs Diagnosis Definition and Differences

2 Detection of Plant Pathogens

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Transcript

Hello welcome to the second talk of this course that is detection of plant pathogens. In the previous talk we have seen that what is the distinguishing characteristics between detection and diagnosis. there we have talked about the various ways of detecting a plant plant pathogen and today we will be talking about the different types of symptoms and signs and they overlap and how to distinguish them in a single plant species so that we can accurately detect a proper disease. Some of the commonly observed Symptoms and Signs for example fungal leaf spots on rice there are two major diseases of rice that causes leaf spots – one is blast and another one is brown spot of rice. In both the cases one can see that dark margins with straw-colored Center spots are seen. In brown spot sometimes these spots are very dark in color and the central portion is also dark brown in color, but the distinguishing characteristic between the dark and light brown spots is that the in brown spot, the spots are relatively smaller whereas, in case of blast the spots are relatively bigger and spindle shaped. Whereas bacterial leaf spots we had see that similar brown spots are present on leaf surfaces but these are angular in nature. Basically, one can very clearly observe that the spots are limited by the veins and that

is why the angular nature of the spots is very clearly evident. Tenderous symptoms which causes by viruses that is known as vein clearing and when banding. in case of vein clearing one can see that the veins are colorless basically the chlorophyll content of the veins is removed or destroyed and that is why the veins look yellow in color whereas, in case of wind banding one can see that along the vein there is a deep green color band is present in comparison to the lamina portion of the leaves where it is lighter in color or light green or yellow in color. This is what we call vain banding and that is how we can differentiate between when clearing and when banding. Similarly, in case of mosaic it is also produced by certain viruses. We can see that there are irregular shapes of green islands in a yellow background and different viruses produce different patterns of mosaic on leaves. At the same time some viruses produce certain typical symptoms which we call it as ring spot whether it may be on fruit or on leaves we can notice that the ring spots are almost circular in nature where the ring region is darker or lighter in nature whereas, the central portion is either darker or lighter but it gives a ring like appearance on both leaves and surface and that is why this symptom is known as ring spot symptom. Then leaf distortion is another symptom that is produced by different types of fungal or bacterial or viral pathogens. In case of peach the leaf distortions can be seen due to a fungal infection where you can see that this is a peach leaf which is distorted. Then, this is a chili plant that is having leaf distortion which we call it as leaf curl then it is caused by a virus whereas in case of banana, leaf swivel leaves are observed in banana plants due to Panama disease caused by a fungal pathogen. So leaf distortion can be another criteria for symptom and it is very distinct from 1 to 1 in different fungal pathogens. Then presence of spores or spores structures on Leafs or other parts of the plant such as presence of white powdery masses in case of powdery mildews, then orange color smut balls that is present on rice in case of all smut, then rust pustules on stem and leaf in case of width, then some leaf spots having fungal fruiting body pycnidia. This black spots on leaf surface is again a fungal infection, then black charcoal

like powdery masses on leaves is again an characteristic feature of sooty mold diseases. So these are fungal spores or structures that are present on leaf or other parts of the plant and they are typical signs of the presence of the pathogen. Then there are bacterial cankers they can have different manifestations for example in case of citrus we can see small cankerous 05:04 legions on fruits and leaves and these are caused by bacterial pathogens, then in certain fruit trees we can see big cankerous growth on the stem portions which is dry in nature whereas, in case of certain other plants where bacterial infections that causes cankers alongwith oozing out of gummy substances which can be another typical signs of presence of bacterial pathogens on different plant parts. Then food decays and rots can also tell us some stories. The patterns of fruit decays and rots for example in case of strawberry, in case of tomato, in case of chilli or in case of mango we can have different types of fruit rots or decays and these patterns tells us about the specific nature of certain diseases and pathogens. Then we have wilts. The wilts symptoms also varies from plant to plant. The wilt symptom initially starts with drooping of leaves, then the leaves turn yellow and finally leaves turn brown in color and the entire plant is wilted. So different plants like cucurbitaceae pigeon pea then 06:19 eggplants chickpea they all have wilt causing pathogens and they affect this plant severely one can have a very clear-cut idea how they wilt symptoms look like in different plants. Then we have another symptom that is we call it as Shoot dieback or blights where the basic nature of the symptom is that the drying of the young in fluorescence or parts of the plants which dries up from the tip and it progresses towards the base of the stem. So this is typically known as Shoot dieback or blights caused by various pathogens. Then overall stunting or Decline can be another symptom of plant disease where we can see that diseased plants have very significantly low growth in comparison to a healthy plant and that is why we call it stunting and then in certain cases the entire plant they die and we call it as decline. Damping- off is another disease symptom caused by certain oomycetes pathogens and where the

seedlings can be died within few hours as the pathogen infects at the base of the seedlings and because of the decay of the base of the plant the entire plant wilts and causes damping -off symptom. So certain symptoms and signs can be observed with unaided eyes and the presence of fungal structures can very well seen without any assistance for example the Sclerotium rolfsi pathogen that causes Collar rot. Here we can see that presence of mustard seed likes Sclerotia that are present at the base of the plant normally with white mycelial traits which is very distinctive in characteristics. A similar Sclerotia producing fungi fungus is known as Sclerotinia sclerotiorum we call it white mold and we hear the symptom is typically manifested by presence of white cottony growths and presence of black colored bigger-sized Sclerotia in comparison to the Sclerotia a of Sclerotinia sclerotiorum rot size which are mustard seed sized but this Sclerotia of Sclerotinia sclerotiorum can go up to one centimeter in size. Then False smut can also very well manifest it in the field condition where initially we see orange colored smut balls which then later turn into a olive green or black colored smut balls which is very distinct from the healthy plants. Then wheat rust pathogens which is manifested in stems or leaves by rust pustules and this is a very clear-cut science of a rust pathogen. Common scab pathogen consists of scabby areas on potatoes and bacterial leaf blight of rice which where the field is very distinctly visible from a distance it looks like blighted or burnt off where the symptom produces along the margin of the rice leaves from the margins and normally on rainy days or in humid conditions we see this type of yellow droplets which later dry and becomes yellow colored balls that are present on the leaf surface and very clearly indicate the presence of the Bacterial leaf blight pathogen on rice plants. Then Fusarium wilt is very clearly identified with symptoms like wilting and the vascular browning of the stems. It's a clear cut indication of Fusarium wilt. this is a pattern we see in tomato, this is a pattern we see in pigeon pea, in both the cases and this is a pattern in chickpea where in all the cases we could very well see the browning of the vascular tissues because

of the presence of the pathogen and its effect. Then bacterial leaf spot whether it is in mango or chilli in both the cases we see that angular nature of the leaf spots which is basically surrounded or limited by the veins of the leaves. In case of chili also the spots are basically angular in nature so that is the typical characteristics of bacterial leaf spot diseases. One can distinguish the bacterial leaf spot and Cercospora leaf spot because as I already mentioned that Bacterial leaf spots the spots are angular in nature whereas, in case of fungal pathogen producing leaf spots such as Cercospora leaf spots the spots are not angular in nature, it is more or less circular and the central portion of the spots are greyish or light brown in color but this definitely they are not angular in nature which is a typical distinguishing characteristic between these two leaf spots. The powdery mildew very well manifested with presence of white powdery masses on leaves on different plant species. Karnal bunt, it's another fungal pathogen. It basically affect the embryo of the wheat kernels and this is a initiation of the disease this is advance stage and this is the most severe form of the disease where w e can see the extent of damage due to Karnal bunt and this is very well or typically it can be identified because of presence of these damaged embryos. Viral diseases can also be very well distinguished because of their symptoms that are produced like Mosaic and Leaf curl. Mosaic it is a green and yellow island which is non uniform in nature, it is scattered whereas, Leaf curl is a very typical symptom where the leaf margins are rolled upside and curled. Nematode pathogens can also be very well distinguished based on the symptoms produced on hosts like Root knots, Soybean cyst and Root nodules can be distinguished from this root knot or cysts by looking at the differences between the symptoms. In case of root knot this is basically the cell division and enlargement that causes by the nematode infection which produces galls on the roots itself whereas, cysts are produced which are extended growth of the plant parts and this ceased basically consists of either off white color or golden in color and they are typically distinguished from Root nodules which are basically produced by root inhabiting

beneficial bacteria like Rhizobium which is simply an outgrowth of the bacterial root system and it is initially pink in nature, later on, it turns into brown but definitely this is having very distinct oozing characteristics from cyst as well as galls. We can use Hand lens to observe certain fungal parts. For example downy mildew where, the leaf spots are basically from the top it looks yellow and only on the undersurface of the leaves one can see the fungal growth by using a hand lens. So particularly this is evident in the morning hours where the moisture level is high in the field conditions one can use a hand lens to see the fungal structures on the below surface of the leaf just below the yellow symptoms on the upper leaved surface. Similarly, we can use hand lens to observe the growth of fungal mycelium on the lower surface of the leaves in case of Late blight of potato and we can very well distinguish the sporangios that are produced on the lower surface of the plants in morning hours when the moisture is present. We can then differentiate Early and Late blight because early blight in potato it has brown dark spots which have concentric rings in the center giving a target board like appearance whereas in case of late blight the brown patches or the dead tissues extends very rapidly and in the morning hours if we see with a hand lens we can see white fungal sporangial growth on the margins of the spot. For bacteria the typical test is Ooze test to distinguish it from a fungal wilt or fungal disease producing agent. So once we suspect a bacterial infection one can take the plants and we can break the 14:56 stem and if we see some mucilage substance coming out between the cut ends then it can very well be established that it may be a bacterial causative agents and if we have access to a glass tube or a beaker then we can put the cotton in a distilled water and we can if we see oozing out of bacteria then we can be well highly confirm that it is a bacterial disease. So we have seen varieties of symptoms and signs that are produced by different types of fungal, bacterial and nematode pathogens of plants and until and unless we distinguish these signs and symptoms which are sometimes very difficult to distinguish from each other. We need a very experienced eye to distinguish them and only by distinguishing these signs and

symptoms we can initially confirm a particular disease or disease causing agent which may be further confirmed through laboratory tests but initial recognition is very very important for distinguishing between a disease causing agent and other abnormalities is due to abiotic factors. So in the next talk we'll be talking about procedures in plant disease diagnosis and I hope you will be able to learn the steps that are involved in the process of disease diagnosis.

Thank you very much.

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3 Procedures in Plant Disease Diagnosis

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Transcript

Hello!! I welcome you to third talk of the week that is procedures in plant disease diagnosis. so we have to follow certain steps and procedures for plant disease diagnosis. It is important to know whether the pattern of the disease that is occurring in a particular field, whether the entire plant is affected, whether certain plant parts is affected, so in considering all these things only we can go ahead for the proper diagnosis of a plant disease.

So first of all it is very important to know whether all parts of the plant or a specific part of the plant is affected by the disease. So it's important to note if the symptoms observed are associated with specific plant parts and is it a wilt observed that is correlated with a disruption of the vascular system or indicated by browning of vascular system or are the roots of the plants abnormal including knots or necrotic lesions observed during strictly on the younger leaves. So whether the symptoms are produced on the roots, or the stems, or the fruits so this is first thing to ascertain, and only because of ascertaining this particular fact which part is affected we can go ahead with diagnosis of the particular disease. Then we have to check the distribution of the symptom where we have to observe the diseased plants which are distributed on the affected

area. We have to see whether distributed uniformly across an area or it is a localized disease and a definite pattern to the distribution is it only along the edges of a greenhouse near open windows or in a low spot of a field or in a planted row only or affecting the plants at random. These are certain criteria that needs to be observed when we try to distinguish a symptom and try to establish the region behind the symptom. Non-infectious problems can also occur in the field because of improper herbicide use or soil factors and they too cause certain types of symptoms on plant parts. So that needs to be distinguished or segregated from the pathogenic ones. A uniform pattern of an individual plant over a large area are usually due to a biotic agents this is a typical pattern where suppose the soil is affected by a certain mineral toxicity or say for example iron toxicity or for deficiency of certain micronutrients like boron or sulphur, it produces a symptom and which is uniform in the entire field. so this is a pattern that needs definitely to be ascertain whether it is pattern may be due to a biotic agent or an abiotic agent and that is why we need to go for checking the distribution of symptom. Then the next thing that we need to consider is that how prevalent is the problem and whether all parts of the plants are affected. Infectious problems occur over time and symptoms progress over time.

So infections normally when it is caused by a biotech agent it progresses over time and it also symptoms are produced slowly from a minimal symptom to a higher severe symptom with time and rarely all plants are affected. It is normally the few plants that initiate with and slowly if the infection is severe then it progresses to other plants or other parts of the plants. Normally, a uniform disease can be caused only if the seed locks are affected by a particular disease then too very rarely we can have hundred-person plants affected by their particular infected seeds. When a problem appears in 100% of plants it's more commonly results from factors like soil conditions, like deficiencies and toxicities as I have already mentioned, adverse climatic factors like, high temperature, low temperature, or draught or toxic chemicals like improper pesticide used air pollutants and so on. So these things needs to be addressed

before we go for final diagnosis of a particular problem. What has been the progression of the symptoms of plant is another questions that can be asked. If the symptoms all appeared at the same time and there has been no further development of symptom this could indicate a possible episodic events such as change in temperature or improper chemical uses. If it is a biotic agent then definitely the disease will progress with time but if the disease progress till a particular time period and after that the disease progression is zero or nil then definitely the cause of the symptoms producing agent is not a biotic one it may be a other measure by biotic or a abiotic factor. If symptoms started in one area and slowly spread to other areas and the severity of symptoms change over time this would most likely due to a biotech agent.

So biotech agent that there will be a pattern of symptom development it will start in one area and that slowly it will progress to other areas and the severity will be changed with time and this is the typical characteristics of a biotech agent. Now check for host specificity, sometimes in some cases, the problem occurs in only one plant species and it's our different plant species are affected. This is a question to be asked. If different plant species are affected this may be possibility of a non-infectious problem which could be related to cultural or environmental problems, but because we know that most of the plant pathogens they are causes disease on a single host and only very limited plant pathogens are there which causes disease on multiple host or related species. For example Phytophthora and Pythium roots rots. More than they can cause root rots in more than one plants species and that we have to look for this type of pathogens also so background knowledge regarding such pathogens is also important when we go for host specificity. If there is more than one species in or plant involved and these plants closely related and they can be infected by a common pathogen, this information is highly essential to establish the biotic or biotic nature of the plant disease. Then it is also important to review the cultural practices and the growing environment of the affected plant or plant areas is that the growers may not be involved in

associated or associate with the problem that is observed in his or her field but maybe it is due to the activities of his or her neighbor which lead to certain problems. So information pertaining to the growing environment is very very important for example change in the environment such as extreme temperature that is freezing or heat, rainfall, prolonged drought etc then site factors such as soil type, drainage problems as well as soil pH should also be evaluated when we go for diagnosis and these are certain aspects that needs to be kept in mind. Cultural and maintenance activities such as application, rate of pesticides, or other chemicals equipment used and unusual occurrence or weather patterns are also important to be considered when we are going for disease diagnosis because they can also have some impact on symptom development on plant species. Careful investigation by the diagnostician is required as someone may have done something improperly and may be unwilling to admit their error. This is also a phenomenon that come across by several growers that sometimes some untoward happening there takes place in crop fields and it is the role of the diagnostician to establish the reason behind it because the person who has committed the mistake is not willing to admit it for the various reasons. Then before going for final diagnosis we need to go for testing of a hypothesis.

So diagnosis can be related to testing of a hypothesis and the hypotheses are generated through observations of the plant, environment and information from the grower. When all information is sources could be consulted to determine already known causes of the disease and disease causing agents. So diagnosis of plant problems is like a detective investigating an assault on the plant species. All clues should be investigated and compilation of the clues ultimately lead to the most accurate diagnosis. Some of the common symptoms may be produced by different types of problems and detailed symptoms and science need to be studied during the diagnostic process for proper diagnosis. So it is simply just like an investigation taking on a crime that has happened in a society the tips and clues or information that

is collected from the crime site or from the crime location is very crucial for establishing the disease causing agent.So initial steps for is to look for signs or biotic causal agents like for example in case of Amillaria infection we can look for white mycelia present in the bark or trees like peach trees, then in case of rust pathogens we can see the stem rust on pustules on wheat plants, then on powdery mildews we can see white powdery masks on the plant structures, then in case of bacterial pathogens along with the symptoms we can go for testing of ooze test that can help us to establish the pathogen associated with the bacterial diseases. So these are the some of the initial steps that we need to look for along with cultural and environmental conditions or information that needs to be gathered before going for final diagnosis. Then initial observations needs to be finally validated or confirmed under laboratory conditions where, the plant material is first incubated in the laboratory, then isolation and anti fixation of biotic plant disease causal agents are done. Then Diagnostics tests for identification of biotic causal agents are done. First we can go for traditional methods and then we also go for modern methods because they are time-saving and they are more accurate and we can go for both traditional and modern methods for establishing the causal agents and diagnostic takes for identification of abiotic disease causal agents is also required to be negated or established in relation to the cases it may be. Modern disease diagnostic methods can be categorized into two methods – the direct method, the indirect method.

In case of direct method we can go for Serological methods like ELIZA, Immunofluorescence (IF) Flow Cytometry Whereas in case of molecular methods we can go for Fluorescent In Situ Hybridization (FISH) method. Polymerase Chain Reaction method (PCR) DNA Arrays. In case of indirect methods we can go for Bio-marker based methods such as Gaseous metabolite profiling, Plant metabolite profiling. Then we can go for plant properties or stress based analysis that is Imaging techniques or Spectroscopic techniques. So depending on the availability of tools and techniques or in necessity of the tools and techniques we can go for either direct or indirect

method of disease diagnosis utilizing various molecular or biomarker for establishing the disease-causing agent. So in this particular talk we have seen that what are the initial steps we need to do. That is first to go for observation of science and symptoms, then we also need to understand the information about the cultural practices it has been adopted, the environmental conditions that has been a face exposed by into the plant system and that's how we can correlate the initial investigations and finally we can go for laboratory methods for confirmation of the initial observation through adopting different methods. So with this we conclude our today's talk and in the next talk we'll be talking about the Quarantine pathogens detection and it's necessity and till then we will see you again in the next talk.

Thank you very much.

Download

PDF: Procedures in Plant Disease Diagnosis

4 Identification and Quantification of Airborne Inoculum

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Transcript

Hello! Welcome to the fourth talk of this week and that is Diagnosis of plant pathogens of quantiryl implications. In the previous talk we have seen the procedures of plant disease diagnosis and today we will be talking about the diagnosis of plat pathogens that have quarantine significance. So, quarantine significance is that, if the pathogen is new to the particular location or to a country then we need to check its spread over a specific geographical location and this is very important for a management of the plant health because of the probable damage it can cause. So for that reassessment is very important and after assessing the risk of the causal organism that can cause certain harm to the plants we here can prevent its introduction and further spread of organisms to other geographical locations. So proper diagnosis and detections are critical for the categorization and assessment of pests that are or may qualify as quarantine pests, and for appropriate application of phytosanitary measures. So, reassessment is very essential from this point of

view.The plant health legislation has both National as well as International regulation framework. The International plant health regulatory framework is set by Sanitary and Phytosanitary (SPS) Agreement of the World Trade Organization (WTO). Member countries have right to take sanitary and phytosanitary measures based on scientific principles. Phytosanitary measures shall be based on International standards. Our guidelines and recommendations developed by FAO Secretariat of the International Plant Protection Convention that is in short known as (IPPC). The scientific principles Phytosanitary measures are based on risk assessment, which includes the evaluation of the likelihood of entry, establishment or spread of a pest or disease within the territory of an importing country. The plant health legislation under the framework of International regulation is very very essential for checking and prevention of a particular disease under the basis of risk assessment.

Diagnosis of Plant Pathogens and Plant Health Regulations – so what is the need of diagnostics, because pest identification is key in the process of pest risk analysis. Pest risk analysis (PRA) consists of three stages: initiating the process for analyzing risk, assessing pest risk and managing pest risk. So these are the three basic stages which are important for pest risk analysis. Initiating the process involves identification of pest as that may qualify quarantine pest or of pathways for which risk analysis is needed, either of these two starting points can involve pests already present in pest risk analysis (PRA) area not widely distributed and under official control as well pest absent from the pest risk area, since both are covered by the quarantine pest definition. So it is important that quarantine pathogen whether it has entered to a particular location or not it is need to be established whether it has already entered to a particular location then it needs to be checked for further spreading on to a new areas. So pest risk assessment determines whether each pest identified or associated with a pathway is a quarantine pest. So, it is very important to establish whether a pest that has entered to a new location is referred to be quarantine pest or not, because certain

other species or related species may be either present on that particular locality or it may not have enough risk to the crops and that may not be the criteria therefore to be qualified as a quarantine pest. So pest risk management involves developing, evaluating, comparing and selecting options for reducing the risk. So pest risk management simply involves developing of and even evaluating, comparing and selecting viable options for reducing the risk. Proper detection and identification is critical for the appropriate application of phytosanitary measures. In addition, diagnostic procedures are needed for determination of pest status in a area, pest reporting and the diagnosis of pest in imported consignments.

Problems for Risk Assessment Posed by Taxonomic Changes: Risk assessment in plant health is challenged by the changes in taxonomy and nomenclature that have taken place in the pathogen. It's a continuous process and we have seen that certain pest or pathogens that were taxonomically placed in other position earlier now has been moved from that particular taxonomic position to a newer position and this has posed a great challenge for the analysis of pest risk because the name or species or the name of the organism is changed from what it is reported earlier. This leads to a reevaluation of pest records and doubts as to whether a regulated organism is actually the one of concern. So this double extra work as we need to reevaluate whether the pest that we are reporting is already reported in that area as a quarantine pest or not so, it is needed because of the changes in the taxonomic position of the particular biologic organism. Pathogens that were previously considered strains or varieties of a single species, have now in some cases been elevated to specific species status. So those varieties or strains that were considered of a particular species has now been transferred to or named and placed in a new species. So, this information is very important for establishing the new pest as a quarantine pest. Further, pathogens recognised as species complexes have been partitioned into new species using molecular methods. So, with the advancement in molecular method it is now possible that some of the pest that were basically or were originally

considered to be species complex is now moved to a new taxonomy species and for that molecular tool has been of great help.

Let us consider some of the examples, say for example Phytophthora Diseases. A pathogen Phytophthora ramorum, it was considered exotic in North America and European nurseries which is responsible for sudden oak disease (SOD) in both USA and European countries. The species had never previously been described in either continent and the distribution was either geographically limited to California of the United States , or clearly associated with the nursery trade in Europe. The high susceptibility of hosts in natural settings and the presence of different mating types in the two continents have made it a big confusion from where this particular Phytophthora pathogen has arrived and further investigations from California forest populations which was able to distinguish from the European nursery isolates and all together three genetically distinct lineages of Phytophthora ramorum was identified and it was found that the North American isolates were distinct from the European isolates and it was also observed that the entry of the pathogen has taken place into the new location through multiple times of trading not only a single time, that is how new strains had been reached into the new location.

Then considering the bacterial diseases for example Erwinia chrysanthemi, it was first included in the genus Erwinia as a pathogen of chrysanthemum, but it was found to be in affective in a variety of plants and that is why 1984 six pathovars has been created that is the dianthicola, zeae, chrysanthemi, parthenii, paradisiaca and dieffenbachiae according to their host specificity.

Futher in 1998 Erwinia chrysanthemi was moved to the new genes Pectobacterium based on 16S analysis. And in 2005 using 16S DNA-DNA hybridisation and biochemistry Pectobacterium chrysanthemi was moved into a new genus called Dickeya, which comprises 6 species , namely dianthicola, dadantii, zeae, chrysanthemi, paradisiaca and dieffenbachiae. So, here we can see the progression of changes of a bacterial genus and species overtime based on different tools and techniques used or differentiating it from the

existing ones. so in 2011, the species Dickeya dieffenbachiae was moved to within the species Dickeya dadantii. And recent analysis we will get the presence of a number of potential in new Dickeya species i.e Dickeya solani, but to date none of these has been officially recognised. So Dickeya species are soft rotting pathogens that causes disease primarily through production of various plant cell wall-degrading enzymes. And description of Dickeya dianthicola follows Pectobacterium parthenii-dianthicola, described on Dianthus species and strains belong to Pectobacterium chrysanthemi biovars 1,7 and 9.

According to several phylogenetic studies including 16S, recA and dnaX sequence analysis, DNA-DNA hybridisation and REP-PCR, Dickeya dianthicola is now considered to be the most closely related to Dickeya dadantii and exhibits little diversity between strains, with no obvious delineation between isolates from different host plants. So we have seen very clear here that how a particular bacterial species has been changed or moved from one particular species to multiple species then t the new genus and then how the new genus is now again been correlated to other existing Dickeya species and probably with time will be able to establish a proper specific identification of this particular pathogens particularly in bacterial pathogen this is causing a big problem.

Similarly, incase of Nematodes the Potato cyst nematodes (PCN), like Globodera rostochiensis and Globodera pallida they are considered to be threats to world potato production and they are able to cause approximately 75% of the total potato ill globally under severe form. So diagnostic of Globodera species based on morphological characteristics of cyst and juveniles is time-consuming and requires specialized taxonomic expertise. Further, visual identification is not always unambiguous and it leads to errors. So biochemical techniques were developed to separate Globodera rostochiensis and Globodera pallida. It distinguish these two species properly as it was not able to do with visual observations. Investigations on differences between European and South America populations of PCN were based on biological and

molecular studies and PCN present in Europe represent only a minor subset of the full biological diversity present in South America and that the range of virulence present in South America is far greater than that present in European PCN populations. So, although it's a global pathogen the biological diversity shows that the South American strains are very highly build in comparison to the European population and the European population represent only a subset of the total biological diversity of the nematode pest that is present in the South America. So with this we have seen that in today's talk that how quarantine pest is important and how risk assessment is need to be done to establish a pest or pathogen to be a quarantine pest and why it is important to check in a particular location and what are the challenges that is associated with categorizing a particular pathogen to be a quarantine pathogen and one of the major hurdle is due to the taxonomic variations that is taking place with time and that is why it is causing more problem to establishing the newer species to a quarantine pathogen with this challenges.

So, in the next talk will be talking about the detection and diagnostic services that are available for helping the farmers and associated growers and we will be talking in detail of the services available at National global and in local level and till then will be having a good time. Thank You very much we will meet again on the next talk.

Thank You.

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PDF: Diagnosis of Plant Pathogens and Quarantine Implications

5 Detection and Diagnostic Services

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Transcript

Hello!! Welcome to the final talk f the week and that is Detection and diagnostic services. In this particular talk will be focusing on the Detection and diagnostic services that has been made available to farmers and growers at various levels and we all know that why this services are required because pest and diseases are responsible about 30% of crop losses annually globally. And Plant clinics have been helping farmers in reducing such losses. So that is why we need to have a proper Detection and diagnostic service system at various levels to help the farmers to reduce such losses. Plant doctors occasionally need expert support from plant health diagnostic laboratories to accurately diagnose plant health problems that are difficult to identify. There are many complex situations prevail in the field situation and many a times visual or initial observation of the disease problem may not lead to accurate diagnosis of the problem and that is why it need to be confirmed at laboratory level and that is how the diagnostic services are essential. Diagnostic services may be present at Agricultural institution level, Private company level, National Level and Global level. So people are developing different diagnostic services at various levels to help the growers minimize their loss.

So, Diagnostic services at agricultural universities if we just see, then globally all of universities they have their own diagnostic services including universities in India so they the growers to identify the cause of the problem and they also recommend the possible remedies for that cause. So at universities or agricultural institutions level diagnostic services has been provided to the growers at various countries. Then there are certain private sector services which also provide diagnostic services to growers, they go for molecular detection of the causal agent and so that they can have proper diagnosis of the problem and then have a remedy for the problem.

Diagnostic services provides at the diagnostic centres – Identification and diagnosis of plant diseases, Screening of water, soil and nursery potting mix for plant pathogens, because plant pathogen may be present in water, soil or even the nursery potting mix. Then Fungicide trials for evaluating chemical and biocontrol products, Seed testing for plant pathogens and Consultancy on planning for reduction in pathogen population then finally Disease control advice. So these are some of the services that are offered by plant diagnostic, disease diagnostic centres and they are helping the growers but there are challenges which needs to be addressed as we know that worldwide there are approximately 500 million are present and they provide food for two-thirds of the earth's growing population. This is highly significant. So, achieving a zero hunger world by 2030 it all depends on by increasing the productivity of these small holder farmers and but their crops face a significant threat. So, yearly an estimated of 40% crops grown worldwide are lost to pest. So these small farm holder farmers they are providing food to 2/3 population of the world and they are facing an endanger of 40% crop loss. So this is the major challenge how to provide proper support to these small farm holders so that they can have proper saving of their produce and therefore they can supply food to the globe. The lack of access to timely, appropriate and actionable extension advice makes it a fundamental challenge to farmers to get right information at the right time to reduce crop losses. So it is

imperative to provide necessary extension service to the farmers initially by diagnosing the problem and then giving the solution to the problem so that they are able to minimize their crop loss and feed the world.

There are certain global solutions one is plantwise. It is basically a global programme led by CABI. It helps farmers lose less what they grow and they are providing great service to the small farming holders, it is kind of a global plant clinic which works in a network mode. It is trained by doctors where farmers can find practical plant help advise. Farmers can visit with their samples and plant doctors can solve their problems and make science base recommendations on ways to manage it. So, it is just like a human clinic where patients visit the doctor. Here isn't of the patients the plants are the patients and the growers bring this suffering plants to the plant clinics were a train plant doctors they diagnose the problem and give solutions to the farmers. The plant clinic network is reinforced by the Plantwise Knowledge Bank, this is a gateway to practical online and offline plant health information, that includes diagnostic resources, best[1]practice, best management advice and plant clinic data analysis for targeted crop protection. So just as the human doctors give suggestions to the patients this plant clinic work in a network mode and they are facilitated by a knowledge bank which help them to diagnose the problem properly and to recommend the best practice for management of the pest that is recommended.

It has a wider context along with giving advice to the small farm holders because it also strengthen the national plant health system by bringing together different people who play a role in delivering knowledge to farmers. It includes extensionists, researchers, educators, policy makers, agricultural input suppliers, farmer organizations, NGOs and many more. So the plantwise is not only plant clinic equipped with plant doctors. This particular platform also strengthening the national plant health system in any countries where it is under operation. Since its launched under 2011, Plantwise has supported over 30 million smallholder farmers across the world with the knowledge they need to lose less of what they grow to

pests and diseases, increasing food security and improving rural livelihoods. So it is in one way it has done a commendable job so far, from 2011 onwards it has provided service to over 30 million small farm holders. Another global service offered by the EPFL and Penn State University and that is known as plant village which is a deep learning app that diagnoses crop diseases. So scientist at Swiss Federal Institute of Technology and Penn State have developed a computer algorithm to identify crop diseases with extremely high accuracy. The algorithm when incorporated in a smart phone app help farmers prevent future food shortages. So, the app is helping farmers to identify or diagnose the problem and also find a remedy based on this diagnosis. The app consists more than 53,000 images of diseased and healthy plants and this was fed into the network and trained personnel to recognize patterns in the data. Based on recognition of pattern of the data the app can or the algorithm can identify a healthy plant or a diseased plant and it can also give a output in the form of a information that this affected plant is because of this particular disease. Neural networks provide a mapping between an input, such as an image of a diseased plant, to an output such as a crop disease pair. So the system works on the same technology like Facebook where it can identify a user by analyzing an uploaded photo. So, in the same way the Facebook works, this particular app also or algorithm also works in a manner.Once the photograph is uploaded it compares it with the existing photographs and then it comes out with an information of possible causal agent of the disease and therefore a viable recommendation can be made out of this output. Within the PlantVillage data set, the model achieved an accuracy rate as high as 99.35% which is very high that means 993 output are accurate out of 1000 images. So this is a very high rate of success and it is been very popular and that is why and is been used globally.

Nuru – is an Artificially Intelligent (AI) in the Plant VIllage system that has been developed to diagnose multiple diseases in Cassava like, fall armyworm, infections in African Maize, potato diseases and wheat diseases. So Nuru is an Artificially Intelligent system,

that is part of the pant village programme that is able to identify certain diseases and particularly helping the growers in the African countries. Then they also have Drones which take Images and videos by cheap, affordable drones so that extension workers in low income countries can rapidly measure disease pressure in smallholder farmer fields. So drones are also providing services to diagnose the diseases in small farm holding farmers and because of their economical nature of the drones it can be used any wherein the globe with a minimal cost.

Then there is a Mobile Spectrophotometry device that has been developed based on nanotechnology and this spectrophotometer has been built to diagnose viral infections in cassava even when the plant looks healthy. Mobile Spectrophotometry can provide rapid disease diagnostics in the field, in real time. So this is another arm or ammunition one can say in the plant village system where it is helping the small farm growing holders to immediately identify a problem and that is how they can take up adequate measures for controlling or managing it. The another app that is developed that is known as Plantix is a free mobile applications which offers farmers and gardners the possibility to receive decision support directly on their smartphone. Due to image recognition this app is able to identify the plant type as well as the appearance of the possible disease pest or nutrient deficiency. So this can be downloaded to any smartphone this Plantix app and one can only take a snap of the disease plant and then with the help of this app its image is compared and then an output is given based on the diagnosis of the possible regions for that particular disease of the plant and the farmers or growers can very well follow the recommendations that has been given by the app. Its born in a Amazon forest Plantix mobile app is helping farmers on three continents quickly identify plant disease using artificial intelligence. Farmers in Germany, Brazil and India using this particular Plantix to upload photos of diseased crops. The images are part of a huge and growing crowd sourced database that is helping farmers to identify, treat and prevent crop diseases. So this is another way, these apps are working globally

to help farmers to identify the problem of the diseased plants and based on this identification a proper recommendation can be given and adopting this recommendation can help farmers to basically grow for proper adequate measures to reduce crop losses. So with we come to an end to today's talk as well as talks of this particular week and in the next week will be talking about conventional disease diagnostic methods that are been adopted in plant disease diagnosis. Thank You so much for being with us for this week will see you in the next week.

Thank You.

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PDF: Detection and Diagnostic Services

PART II

WEEK 2: CONVENTIONAL DIAGNOSTIC TECHNIQUES

1 Koch's Postulate for Biotroph and Necrotroph Pathogens

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Transcript

Hello! I welcome you to the second week of the course Detection, Diagnosis and Management of Plant diseases. In the first talk of this week we'll be talking about Koch postulates for biotrophic and Necrotrophic pathogens. We all know that Koch's postulates has been given by Robert Koch while he was working with the etiology of cholera and to ecologies but the postulates he developed for establishment of the microorganism associated with cholera and tuberculosis convey extended for other plant pathogens which are microbial in nature. So, there are four steps basically in Koch postulates. In the first step there is a must association of microorganisms with all individual plants affected by the disease but those microorganisms should not be associated with healthy plants. In the second step the microorganisms should be isolated from the disease plant and grown in culture. This is very important and the third step is when introduced into a healthy plant the cultured microorganism should cause disease. And the fourth postulate is the microorganism must then be re-isolated from the experimental host and found to be identical to the original

microorganism. So in the four steps it is essential to carry out for most of the microbial pathogens which are associated with plant diseases. But sometimes there are certain pathogenic causes or the host properties that is a source difficulties in establishing the cause postulates and that is why the disease should be produced in a new host but it is not a must, because there may be certain asymptomatic carriers, or the host immunity factor, or the genetic resistance, that are possible and these factors may not allow development of the disease even though the pathogenic micro is inoculated to an experimental host. So Koch postulates for biotrophs can be different from Koch postulates from microtrophs, particularly in the steps of inoculation and isolation of the pathogen.

So Koch postulates for stem rust of wheat can be taken an example for biotrophs where, wheat seeds are of a susceptible cultivars sown in the pots and the seedlings are grown until the first leaves are fully emerged. Then during inoculation leaves should be rubbed gently between the moistened fingers so as to remove the waxy layer of the surface of the leaves which hinders penetration of germ tube of the pathogen spores. Then spores should be suspended in distilled water and with a drop of Tween 20 and the spore suspension should be spread until runoff with an atomizer. The seedlings of inoculated and incubated a relative humidity of about 100 percent and the temperature should be of around 22 degree Celsius in a plastic chamber for at least 24 hours.

This is important for giving the proper facility for development of the spore germination, germ tube development and penetration . By the rust pathogens transfer of seedlings to a greenhouse bench should be done and the temperature should be maintained at around 18 to 27degree celsius and at least it should be kept for two weeks for development of the symptoms. Samples with viable spores be selected to multiply in a columns for generation of monopustule isolates. So the inoculation with monopustule isolates is important because if it is not monopustule then there may be a mixture of races that may be present in the same leaves and it may

not lead to a differentiating response in the host plants. So monopustule isolation before sporulation should be done for spore suspension preparation in case of wheat rust pathogen so that a clear-cut distinct Koch postulate could be proved. Then the question arises how to select a monopustule isolate for identification of races. The leaves with monopustule all infections should be identified prior to sporulation and then isolated because, once the sporulation takes place then there may be a mixture of spores that may be present in the suspension. In case where the pustules are aggregated and no isolated past will occur, inoculation is repeated on the susceptible cultivar until separate pustules are developed and monopustule isolates are generated. As long as we get monopustules we can keep on repeating spraying of the monopustule on wheat cultivars and a susceptible cultivar so that we are able to generate a monopustule and develop spore suspension from the same single monopustule for identification of wheat rust pathogen races. The generated monopustule isolates are then further multiplied on a susceptible host until sufficient urediospores are collected for differential host test. So this is very important to generate monopustule for differentiation of a race of wheat rust pathogen. Then the cost postulates for necrotrophs may be slightly different from biotrophs. Let us take the example of Alternaria species where Foliar spray of the spores is also done but after culturing of the pathogen on an agar plate. So in case of Alternaria the pathogen should be isolated and cultured on agar medium at the very beginning. Then once the sporulation takes place there distilled water should be placed on the surface of the plate and then slightly vortex thing should be one. After that the spore suspension should be decanted on a beaker and then it should be collected in a hand atomizer and then healthy plants are sprayed till water drips off from the leaves. Then the leaves are covered with a plastic bags for at least 24 hours and allow the plants to grow in a glass house /net house till the symptoms are developed. So in this way the Koch postulates are also proven in

necrotrophic pathogen but isolation of the pathogen as well as handling of spore suspension from different sources matters.

Similarly, another Necrotrophies the soil borne pathogen that is Fusarium. Here the like panacotic pathogen is not a Is not leave inoculated rather it is soil drenched. Spores of the Fusarium were collected in a similar way just in the case of all Alternaria species but after collection in the beaker the 30 to 50 ml of the spore suspension is then drenched in the pot around the plants and the pots are kept for appropriate temperature for development of the disease and development of the wilt Symptoms. So in case of Fusarium we have seen here that instead of spraying of Spore suspension the pathogen is soil drenched around the plant in the pot Soil. Similarly, in case of collar of pathogen that produces mycelia and sclerotia by Sclerotium rolfsi, the application method is inoculation of Mycelia and sclerotia directly into the soil. The pathogen is isolated from an infected plant part it may be a stem or colaor and obtain pure culture on a suitable agar medium. Then inoculate the fresh mycelium disc on cereal grains priorly autoclaved and packed in a polythene bag. Allow the pathogen mycelium to grow and develop sclerotia. Then take out cereal grains colonized by the Sclerotium rolfsi pathogen mycelium as well as sclerotia of about 5 to 10 grams and the care should be taken that adequate soil moisture is already prevailed and the 5 to 10 gram grains should be inoculated into topsoil in pots and mix with the topsoil Thoroughly. Then allow the plants to grow till symptoms are developed. So again this is another necrotrophic pathogen but the method of inoculation for testing Koch postulate is different. Here the pathogen is cultured on cereal grains and the colonized grains with the pathogen mycelium and sclerotia is then inoculated into topsoil of the plants and then allowed to develop infection and symptom in the pot till the appropriate symptoms are observed. So here we have seen that for different pathogens the proving of Koch postulates may have some differential steps to be taken care of so that appropriate disease symptom is generated in the host plants to prove the pathogen to be associated with the

disease-causing agent. So with this we come to an end of today's talk and in the next talk we will talk about the concepts of pure culture and selective media and, we will see their how pure cultures of fungal and bacteria is done and what is the role of selective media in obtaining the pure culture of the plant pathogens.

Thank you very much.

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PDF: Koch's Postulates for Biotropic and Necrotrophic Pathogens

2 Concepts of Pure Culture and Selective Media

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Transcript

Hello!! welcome to the second talk of the week that is pure culture and selective media. Pure culture is very very essential because it gives us an opportunity to identify the disease causing agents and help us to establish the relationship between symptom development and disease causing agent.

So it is very important to understand how pure culture is done for Fungi and Bacteria and let us see what are the major steps that needs to be follow for pure culturing of fungi that causes plant diseases. There are two basic methods that are used for pure culturing of fungal pathogens of plants. First one is: Single spore isolation method and the second one is Single hyphal tip method. In the Single spore isolation method, this method is applied to those pathogens of fungal origin which produce spores and which are coloured and bold. So these are some important criteria which are basically required for following this particular Single spore isolation method. The procedure involves that we normally take 3 tubes of plain agar which is melt and cool them to 50o C then transfer a loopful of spore suspension of the mixed to the first plain agar tube

with an inoculation needle, then we should shake the suspension agar medium for uniform suspension of the spores, then transfer a loopful of the first dilution to the second tube and shake thoroughly. So this the step it is done here, likewise prepare third dilution as well so, we should go for second and third dilution and then pour the media with diluted spore into three separate petriplate and allow to solidify. Then after dilution we should pour the medium into petriplate and then we should allow the other medium to solidify. We should observe petriplates under low power objective of the microscope and locate isolated single spore. The single spores that are isolated on the other medium should be located and the single spore should then be transferred to PDA slant for obtaining a pure culture. This is the basic method for isolation of pure culture of fungal pathogens through single spore isolation method. Then second method is Single hyphal tip method – this method is employed for purifying fungi which either do not produce spores or produces small and hyaline spores which are difficult to isolate in a single state isolation method. The procedure is by using a cork borer we should take a disc of fungal colony and place it in the middle of a plain agar plate and incubate for 1 to 2 days. So once it is placed and incubated for 2 days then place the petriplate on compound microscope and locate a hyphal tip using the low power objective. Then this plate can be now placed in a microscope and we can see the hyphal tips under low power objectives. Then with help of a cork borer we can cut this single hyphal tip and remove it and place it into a PDA slant and maintain it as a pure culture. So, this can be transferred to a PDA slant and then it can be a pure culture from the pathogen. So, this is another simple method which we call it as single hyphal tip method for isolation of the plant pathogenic fungi.

Yeast purification can also be done but in a different way since yeast are single celled eukaryotic microorganism and produces slimy white soft colonies resembling the bacteria. The will not produce mycelial filaments. And that is why Purification can be done like that of bacterial cultures using Streak plate method pour plate method and spread plate methods. So yeast cells a separated in the

similar way as in the case of bacterial pathogen. So, the methods used to isolate bacterial pure culture are also mostly the Streaking or Plating and Dilution or Plating method. In a Streak plate method what we do is we take a inoculation loop and then take a loopfull of bacterial suspension and then streak on agar plate in this direction followed by another direction, opposite to it, then another direction and then another direction. So, by doing this what we do is that we dilute the original pore suspension to an extent that finally it gives rise to scattered single spores and this spores when incubated on suitable agar medium they develop into a single spore colony. So this is the Streak plate method for isolation of bacterial culture.

In the Spread plate technique – we take the bacterial suspension and then take 0.1ml of the bacterial suspension on agar plate and then with the help of a sterilized glass rod we spread the spore suspension to rectangular suspension and then allow the plate to incubate for 24 to 48 hours. After 24 to 48 hours the spores that are separated by this method give rise to single spore colonies and these colonies can now be again transferred to slants for maintaining as a single spore isolation of the bacterial pathogen.

Then the concept of Selective media comes because all fungi and bacteria is difficult to separate from a common medium. So Selective media are used to isolate selective microbes whether this fungi or bacteria that causes certain plant diseases in a different way. So Selective media are used for the growth of only selected microorganisms. For example, if an microorganisms is resistant to a certain antibiotics, such as ampicillin or tetracycline, then that antibiotic can be added to the medium to prevent other cells, which do not possess the resistance, from growing. So use of antibiotics can be part of selective a microbial growth because it suppresses the sensitive microbes and it only allows the microbes that are tolerant or resistant to these antibiotics. So, examples of selective media include:

-Eosin methylene blue that contains dyes that are toxic to Gram-positive bacteria so, it is used for selective and differential medium for coliforms bacteria.

-YM(yeast extract, malt extract agar) has a low pH, deterring bacterial growth.

-Similarly, MacConkey agar is Gram-negative bacteria.

-Whereas, mannitol salt agar is selective for Gram-positive bacteria and differential for mannitol.

-Then Sabouraud's agar is selective to certain fungi due to its low pH (5.6) and high glucose concentration. So by varying the composition of the medium we can develop certain selective media that is suitable for isolation of certain specific microorganisms whereas other microorganisms they don't grow profusely on this medium and that is why they help us in selecting or isolating a specific micro organism based on selective media.

Actinomycetes have different types of selective media for their isolation like:

Yeast extract malt extract agar (ISP2)

Actinomycetes isolation agar (AIA)

Arginine Glycerol (AG agar)

Glycerol asparagines agar (ISP5)

and so on. So these media are very specific for growth and development for Actinomycetes very specifically when it was isolated from a mix cultures of other fungi bacteria. Then for a plant pathogenic fungi like Phytophthora we have selective medium like : V8 juice agar medium or Rye A agar medium that suitably used for isolation of phytophthoras species and suppressing other pathogens of fungal or bacterial origin.

So, here we have seen that how pure culture is obtained for fungal and bacterial cultures and what is the concept of selective medium, why they are used? And we have also used examples of selective medium, why they are used and how they are used for isolation of certain specific microbes that may be a causal agent for certain plant pathogens. So, with this we come to an end of lecture number 2 of this week and in the next lecture we will talk about microscopic techniques and straining methods for diagnosis of plant pathogenic fungi and bacteria.

Thank you very much.

Download

PDF: Pure Culture and Selective Media

3 Microscopic Techniques and Staining Methods

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Transcript

Hello!! Welcome to the third topic of the week that is Microscopic techniques and sustaining methods. Microscopy is an important element for plant diagnosis because we can see directly the micro organisms associated with disease plants and it help us to establish a initial etablishment of the relationship between the observe microorganism along with the symptoms it is produced in the host plants. So for microscopy we need certain training procedure as well and we will be looking into those aspects that are used for common plant pathological work. So, broadly Microscopy can be categorized in 2 broad groups: one is Optical microscopy and the other one is Electron microscopy. In Optical microscopy the conventional light microscopy, Flurescence microscopy and confocal microscopy comes into existence. Otherwise Optical microscopy is also known as light microscopy and it involves the usage of visible light and one or lens to produce an enlarged image of an object that is placed in the focal plane of the lens. This can either branch off into transmission, where the beam of light passes through the sample. There are many applications of Optical

microscopy such as in microbilogy, nanophysics and biotechnology but in plant pathology it is mostly known for using diagnostics of infected plant tissues. So, Light microscopes normally have 3 types of magnifications:

The low power (10X)

High Power (40X)

Oil immersion (100X)

And they have the capacity to magnify 10 times, 40times and 100 times accordingly. So, Light Microscopy or Optical Microscopy can be of two types: Normal Binocular Microscope and Inverted Binocular Microscope. In Normal Binocular Microscope the light passes from the bottom through the specimen whereas, in case of Inverted Binocular Microscope the light resource is present on the top and the specimen is present in the base were the light passes through from the top to bottom.

Incase of Electron microscopy: The most common electron microscopes are Scanning electron microscopy (SEM) and Transmission electron miscroscopy (TEM). This is a form of microscopy that uses electron beams to create an image of the object being used. They have a much higher magnification than light microscopes. This allows us to see smaller specimens with greater detail. The resolution is abled to be increased because as the electrons travel faster their wavelength becomes shorter so there is a direct correlation between reducing wavelength and increasing resolution. So, this is the reason because the electrons move much faster, so the wavelength of electrons are very small and because of that it is able to have better resolution from the electron microscopy.

There are two types of electron microscopes that are used one is Transmission and the other one is Scanning electron microscopes. TEM involves shooting a high voltage beam through a thin layer of specimen and gathering information about the structure whereas, SEM in contrast produces images by detecting secondary electrons that have been emitted of the surface due to excitation by the primary electron beam. So, the basic principles it is of two distinct

types – in case of transmission Electron moves through the thin layer of specimen whereas in case of SEM the image is gathered from the detecting secondary electrons that have been emitted from the surface of the specimen. Here is an example of comparative microscopy of light of optical microscope SEM and TEM. Here you can see that rust fungal pathogen spore that is germinating and this is on the surface of leaf under light or optical microscopy. This is the image generated under scanning electron microscopy and whereas, this is the image generated on transmission electron microscopy where this is the fungal spore, storium and this is the hastorium. This structure can be detected through three sectioning of the fungal infecting sight and this is how the images are created by optical. Light microscopy, scanning microscopy and transmission electron microscope.

Staining of fungal structures is very important to observe. As we have seen in the previous slide how fungal spores we can be visualize under light microscopy. So, Lactophenol Cotton Blue Stain is the common stain that is used for staining the fungal structures. So, it is formulated with lactophenol, which serves as a mounting fluid and cotton blue serves as a stain. The organisms suspended in the stain are killed due to the presence of phenol and cotton blue is an acid dye that stains the chitin present in the cell walls of fungi. That's how we are able to see the fungal structures under light microscopes in a very clear cut manner. So in totality Phenols that is used it kills all the living organisms like fungal spores, mycelium then the Lactic acid that is used as a Lacta phenol – preserves fungal structures and Cotton blue – it is the stain that stains the chitin of the fungal cell walls. So, that's how we are able to visualize the fungal structures with the use of Lacto phenol cotton blue.

So how to prepare a Lacto phenol cotton blue solution (LPCB)- so these are the ingredients : Cotton Blue, Phenols, Lactic acid and Distilled water. An the stain is prepared over two days. On the first day one has to dissolve cotton blue in distilled water and leave it overnight to eliminate the insoluble dye. On the second wearing gloves add the phenol crystals to the lactic acid in a glass beaker and

place on magnetic stirrer until the phenol is dissolved. Then finally add glycerol. Filter the Cotton blue and distilled water solution into the phenol/glycerol/lactic acid solution. Mix and store at the room temperature.

So in brief, this is the procedure how lactophenol cotton blue (LPCB) is prepared over two days time and this is the most common staining agent that is used for staining fungal pathogens. So, normally observing fungal spore on glass slides is done at low power and high power and Hyaline spores are basically stain with lacto phenol cotton blue and we can see the spores very clearly in comparison to the same spore without staining . Coloured spores like in case of bicolories or Altrneria normally no staining agent is used and they are still observed under low and high power because of their own inheriting colours the staining is not required for visualizing of such fungus under microscope. Then there is another technique for observation of spores on intact leaf surface and what we need to do here is that we need to clear the chlorophyll content of the leaves on which the spores are present.

So, the leaf simple Leaf clearing technique involves: Ethanol and acetic acid solution in the ratio of 3:1 and it is to help in removal of chlorophyll from the leaves. After clearing the leaves staining was done by Coomassie blue for visualization of pathogens like Erysiphe pisi that causes powdery mildew on different pea leaves. After staining, leaves are kept on glass slide and observed under compound light microscope for conidial presence and their germination. So leaf clearing technique is used mostly to remove the chlorophyll content of the leaves followed by staining of the fungal structures like spores or germinating spores so that we can see the association of fungal spores with the particular host plant and its development on host plants.

Then Gram staining of bacteria – it's an important staining procedure for differentiating gram positive bacteria from gram negative bacteria. Any isolated bacteria from infected plant samples can be first differentiated with the help of this gram staining procedure. So the procedure comprises of preparation of a bacterial

smear on a glass slide, then we should go for air drying of the bacterial smear. Then it is stained with crystal violet and it is done for approximately 30 seconds, then bacteria gets stained with bluish purple colour then it is washed with water followed by adding iodine staining solution, it works like a mordant and it forms a complex with the crystal violet and gives a crystal violet iodine complex. The, this is subjected to alcohol treatment for few seconds to a minute and this alcohol treatment can differentiate between the gram positive bacteria gram negative bacteria. In case of gram positive bacteria they will retain the crystal violet colour and they can be confirmed as gram positive bacteria. The appearance will be like this, it will be kind of bluish purple. Then, some of the bacteria will be destained after the alcohol treatment and they can be done counter stain with another stain that is Safranin and then the colours of the bacteria will turn into pink and this will confirmed at those bacterial cells are gram negative in nature and their appearance will look this under normally oily immersion. So this is how we can differentiate the bacterial pathogen that is isolated from an infected plant and they can be differentiated on the basis of gram staining whether the pathogen is gram positive bacteria or gram negative bacteria and then, visualizing the structures and other things.

So, in short we have seen the common microscopic techniques that are used for diagnosis of plant pathogens that is associate with plant diseases and then simple and staining methods that are most commonly used for staining of fungal pathogens and bacterial pathogens for differentiating them and correlating them with the associated disease symptoms. So, with this we have come to an end of the topic microscopic techniques and staining methods. In the next topic we will be discussing about diagnosis seed borne pathogens. Till then..

Thank you very much.

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PDF: Microscopic Techniques and Staining Methods

4 Diagnosis of Seed Borne Pathogens

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Transcript

Hello!! Welcome to the fourth talk of the week that is detection of seed borne pathogens. Seed borne pathogens we now that it has greater impact on agriculture as well as seed industries and detection of seed borne pathogens is very much essential as the seed borne inoculums me give rise to severe disease investigation in the field condition and severe loss to the growers. There are conventional methods as well as molecular methods that is deployed for the detection of diagnosis of seed borne pathogens. Some of them are like:

Blotter method
Agar method
Paper towel method
Embryo Extraction method
De-hulling and embryo extraction
Extraction and Agar plating method
Extraction and Polymerase Chain Reaction method
ELISA method

These are some of the common seed borne methods applied for seed health testing for detection and diagnosis of seed borne pathogens. Let us talk about the Blotter method: Say for example

Alternaria dauci that causes disease in Daucus carota (carrot) and it's a seed borne pathogen. It can be diagnosed with Blotter method. In this particular method we take 3 layers of blotting paper on both the lids of petriplate and the we moisten it and then we place the seeds at uniform distances and then we incubate it for period and then once pathogens grow outside the seed we can visualize the pathogens under microscope at different magnifications. And then by looking at the morphology of the fungal pathogen associated with it we can very well identify the pathogenic nature of and the pathogens associated with the carrot seeds. So, in short three layers of 90mm filters are placed in both the lids after soaking with sterilized distilled water. We have to drain away the excess water.

Then we should place atleast 10 seeds on the plate, evenly placed, on the surface of the filter pare, then incubate it for 3days at 20oC in the dark. Then transfer the plate to freezer and maintain a temperature of -20oC for 24 hours and after freezing incubate for 6 days at 20oC with alternating 12hour period of darkness and near NUV lights and plates should be approximately 25cm below the lights and should not be stacked. Then we can examine as we have shown in the previous slide, the fungal spores or fungal growth on the seed surface and we can establish the relationship between the inherent thing pathogen like fungi that is on the carrot seeds following this particular method.

The same Blotter method is also used for detecting seed borne pathogens such as Alternaria radicina in carrot, Botrytis cinerea in sunflower seeds. Then Alternaria radicina in carrot can also be established through malt agar method. Malt agar method can also be diagnosed through Leptosphaeria maculans and Plebdomus biglobosus in Brassica seeds, then Ascochyta pisi in Pisum species and the method is basically Aseptically place around 10 seeds evenly spaced on the agar surface of malt agar plate. Then incubate it for 10days under 20oC with altering 12hour periods of darkening and near UV light (NUV). The similar way plate should be approximately 25cm below the lights and plate should not be stacked. Subculture of reference culture to a malt agar plate at the same time of the

seeds are plated and incubated in the test plates so that we can have a comparative idea between the actual culture and the fungal growth that is taking place on the seed surface. Then examine the plates visually under stereoscopic microscope to establish the morphological characteristics of the associated fungi.

Colletotrichum lindemuthianum in bean then Bipolaris oryzae in rice these are some of the other pathogens that are also used in agar plate method for detection as seed borne pathogens. Then the next method is Rolled paper towel method where seeds are placed between two paper towels and paper towels were moistened and the seeds were allowed to germinate and grow as seedlings and by looking at the seedling health one can establish from the germinating seeds of infected and healthy seeds then looking at the fungal spores or fungal colony strata associated with the infected seeds to establish the relationship between fungi that is causing certain seed borne diseases.

Embryo Extraction method – it is used for Ustilago nuda in case of barley. So the method basically as such we have to place the seeds 1 litre of freshly prepared 5% aqueous solution of sodium hydroxide and maintain at 20oC for 24hours. After soaking, the entire sample should be transferred to a suitable container and washed in warm water to separate the embryos, which appear through the softened pericarps. Then collect the embryos in a sieve of 1 mm mesh. Additional sieve of larger mesh can be used to collect pieces of endosperm and chaff. Then transfer the embryos to a mixture of equal quantities of glycerol and water in which further separation of embryos and chaff can be made and transfer the embryos to a beaker containing 50ml of lactic acid solution and clear them by maintaining the lactic acid solution at boiling point for approximately 5min in a fume cupboard. Then transfer the embryos to fresh glycerol for examination. The scutellum becomes more transparent when embryos are left in glycerol for 1-2 hours making the examination much easier. So, this is how we can go for Embryo Extraction method for detection of Ustilago nuda in barley seeds.

Then we have to examine it under microscope for presence of the Ustilago nuda pathogen.

Ustilago nuda in barely seeds can also be done by dehulling and embryo extraction method. Here, the embryo extraction method is the same but the dehulling method has to be preceded the embryo extraction method. In case for dehulling place the working sample in glass beaker with 25-37% sulphuric acid until the seeds are covered. Incubate in an oven at 75oC for 50min or until the seeds turn a medium brown colour. Carefully pour of the sulphuric acid solution, rinse seeds by pouring water into the beaker, gently mix and pour off the water and add new

water and remove loosened hulls by stirring robustly with a rod. Remove hulls by carefully removing the water. If hulls remain, add new water and either use an hand mixer at low speed or continue stirring. Repeat the procedure until all hulls are removed. Be careful not to lose any kernels i.e. seeds without hulls. So, after dehulling we can go for embryo extraction method as it is mentioned earlier.

The next stage Extraction and Polymerase Chain Reaction method – So, this is used for detection of Xantomonas campestris, pathogonas campestris in Brassica seeds. Here the pathogen is first cultured on petriplates and then DNA is extracted from the bacterial cultures and PCR amplification is done using specific primers for detecting the pathogens. Presence of specific bans on the cultures confirms whether the pathogen is of the targeted ones or and other microbes that is associated with. The detail about Polymerase Chain Reaction method will be discussed in subsequent talks. Similarly, Elisa method is used for detection of certain viral diseases of that are seed borne in nature for example squash mosaic virus, cucumber green mottle mosaic virus and melon necrotic spot virus in cucurbit seed.

So, another test that is known as Grow-out test. So this infected seeds are grown on sample pots and they are allowed to grow in a way that disease symptoms are manifested on the leaves. So, this is another confirmatory test which we call it as Grow-out test. It can be also perform for Squash mosaic virus where the symptoms

are very much evident after growth of the seedling to a certain stage and by doing this test by looking at the symptoms one can confirm whether the pathogen is associated with the seeds or not. So, these are some of the basic methods for detection and diagnosis of seed borne pathogens and with the time different molecular tools have been deployed for detection of seed borne pathogens more accurately along with the races and strains that are associated with the disease seed lot and finally we can conclude that with this methods we can certainly able to establish a seed lot whether it is affected or not with certain fungal bacterial or viral pathogens.

Thank You very much.

Download

PDF: Diagnosis of Seed Borne Pathogens

5 Disease Diagnostic Kits

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Transcript

Welcome, to the last talk of the week that is disease diagnostic kits. We have seen that in the previous talk that we have conventional methods as well as certain molecular methods for detection and diagnosis of certain plant pathogens whether it is seed borne in nature or that is associated with any sort of plant diseases, but with the advancement in plant biotechnological tools and techniques people are now able to have their Disease Diagnostic kits which are very handy and ready to use and they are able to detect the possible causal organisms of the plant diseases very efficiently in a short span of time.

So biotechnology has allowed the development of Diagnostics which is assisted farmers worldwide in managing different diseases affecting their crops. New diagnostic techniques require minimal processing time that is very important and they are more accurate in identifying the pathogens. So this is how this diagnostic kits are becoming popular day by day. This Diagnostics are based on rapid detection of proteins or DNA that are specific to each pathogen, disease or condition. So they are designed to detect different pathogens or different conditions separately. Some procedures

require laboratory equipment and training while other procedures can be performed on site by a person with no special training. So it is the device that gives us the indication or they tells us whether that particular pathogen is present or not on the sample. So there is no need of highly trained personnel to be involved in such diagnostic kits, associated with plant disease diagnosis.

So plant disease diagnostic basically based on the principles of protein or DNA based methods and there is a broad range of ELISA that is protein based diagnosis based method that is available. ELISA kits are available for plant pathogen detections and with very high test performance characteristics to allow accurate rapid simple and high-throughput identification of the organisms that causes plant disease. The immunological technique based on ELISA kits offer considerable advantage over traditional diagnostic methods and PCR-based techniques as these Eliza kids are easy to handle and very fast to give the results, within few minutes one can have the results in hand. In addition a wide range of ELISA-based rapid test strips are also available with visible color chain signal by using lateral flow devices which are diagnosed for on[1]site robust and fast detection of plant pathogens by even unskilled personnel.

That is the beauty of this ELISA kits and they are able to detect plant pathogens very efficiently. Some of the examples of diagnostic techniques like (enzyme linked immunosorbent assay) kits. So, ELISA kits are based on the ability of an antibody to recognize a certain protein substance or antigen associated with a plant pathogen. So pathogen specific antibodies are developed and these antibodies are used for rapid detection of pathogens in this ELISA kits. The kits are very easy to use and can be used in field to disease only in detected disease only in 5 minutes. In addition they do not require sophisticated laboratory, equipment or training. There are already numerous ELISA test kits available in the market to detect diseases of root crops, ornamental crops, fruits and grains and vegetables. So technically the method involves collection of infected plant 04:01 part and this is then macerated in the tube with the solution that is buffering solution and the resultant solutions

a suspension is then pipetted it out and it is then a few drops are placed in the well of the diagnostic strip and appearance of a single line gives the positive indication of the pathogen because this line is basically, it is the antibody and once the antigen that is protein, specific protein is of the pathogen moves through this lateral flow device and reaches the antibody then there is development of color and in case, if pathogen is not present then there is no development of such strip success line is observed. And, this is how detection of pathogens specific in nature can be obtained by using these strips and even the people with little knowledge can establish whether the plant sample is having that particular pathogen associated or not. So for different pathogens we have different diagnostic or, little flow devices such as for Erwinia amylovora Phytophthora then Phytophthora species Raistonia solanacearum and so on. So there are different kits available for different pathogens but more or less the basic technology is same for detecting all the pathogen except for the antibody used for different strips are different for detection of different pathogens. Then there is another technology known as ImmunoComb, this is from agdia company and it joins together the most requested Agdia test for plant virus detection because the grower can test for Cucumber mosaic virus (CMV), Tomato spotted wilt virus (TSWV), Impatiens necrotic spot virus (INSV) and Tobacco mosaic virus(TMV) virus all at one time. The procedure ImmunoComb involves the samples are grounded in a special bag that allows for easy extraction the comb of four test strips is then easily placed into the sample and the test takes just minutes to form clear yes or no results. So in the sample four different strips are inserted and if any of this four pathogen is present in the sample then one of the strips will give color indication for presence of the one specific viruses and that's how within minutes one can confirm whether the particular virus is present or not.

Then there are direct tissue blotting method – in this technique utilizes specific antibodies to detect the presence of plant pathogens. These tissue samples are pressed to draw out proteins onto a special paper and the antibodies are added to the sample.

The color inducing reagent is added afterwards had to react with the antibody pathogen complex. And color reaction indicates a positive result and pinpoints the location of the pathogen in the diseased tissue. So what is done in the direct tissue blotting method is that one has to squeeze out the juice of the infected tissues and it should be spotted on a specific paper and then antibody is added along with the colouring agent. If the color is produced then it can quickly detect whether that particular pathogen or again switched antibody is raised is present or absent. Then DNA and RNA probes – this is another set of tools that can be used in plant disease diagnostics using nucleic acid whether it is DNA or RNA as probes. These probes are fragments of nucleic acid arranged in sequence complementary to that of DNA or RNA of the pathogen. Because of the sequence complementation each other the probes can be used to identify specific Disease. So pathogens DNA or RNA specific probes can be applied to detect or action of the pathogen DNA or RNA in the sample and because of their complementary they will emit different types of signals and these signals will then confirm whether that particular pathogen is present or absent. Then Squash blot method – this is another method where the tissue from an infected plant is " squashed" into a specialized piece of paper we call it a membrane. Then the membrane is treated with a probe that can bind with the DNA or RNA of the plant pathogen suspected to be in the tissue. Binding will occur when complementary sequences are present. After adding several more substances to the membrane a color reaction indicates that the probe and the pathogen DNA/ RNA have bound to each other and the disease is present. No color reaction means the test is negative for the particular disease. So this is another method very rapid and quick method where the tissue is squashed into a membrane and then DNA or RNA probe is added to the membrane. The pathogen complimentary pathogen DNA or RNA is present in the squashed area then the DNA probe will go and bind with it. Then Polymerase Chain Reaction in short we call it PCR. It is used nucleic acid probes to detect the presence of a pathogen. But this is lot more sensitive compared to other techniques as PCR can

detect very small amount of pathogens genetic material or sample and amplify certain sequences to a detectable level. PCR can be used to detect the presence of pathogens in air, soil and water. Spores, especially those produced by fungi are the primary source of infection to initiate epidemics. So farmers can therefore keep track of the pathogen and apply the necessary control to prevent the spread of the disease. So it's another highly very sensitive instrument polymerase chain reaction and this technique is used to detect even very small amount of pathogen sample that is present either in air, soil or water so, that the farmers can be aware of the trick that is coming in short time. So Pocket Diagnostics have developed rapid test strips for plan disease in the diagnosis. And this lateral flow rapid test strips normally are available for detection of different plant pathogens.

We need just need to cut or tear sample into small pieces and put into bottle containing buffer and small bearings. Here instead of using a rod the tissue is macerated by using ball bearings by shaking it firmly for 30 to 60 seconds to break the sample tissue and allow the liquid to settle down. Then draw the liquid with the help of a pipette. It should be avoided the debris and air bubbles. Keeping the test device level add two drops of the sample well into the device. Here they have added the pipetted out the sample two drops and the results can be obtained within 10 minutes as we have discussed earlier were in a positive interaction give rise to a new signal that is a new band on the diagnostic kit.

Then PCRD is another technology where one can have rapid detection of plant pathogen following PCRD methodology. After the PCR polymerase chain reaction for detection of amplification we have to go for agarose gel electrophoresis but this particular diagnostic kit can help us to avoid that gel electrophoresis and thereby reduce the time to a significant amount for detection of the plant pathogen that is associated with the disease sample. So in PCRD is just a simple and rapid alternative to gel electrophoresis that can be performed in a matter of minutes without the need for expensive equipment or exposure to intercalating dye and UV light.

These are also very harmful to human being and in this with the use of PCR D we can help us how to avoid this dyes and UV light PCRD is a basically a nucleic acid lateral flow amino acid suitable for use with PCR, LAMP technology then, RPA technology as well as HDA technology. The format of PCRD is suitable for use in both high-throughput laboratories as well as small field based laboratories.

Then what are the benefits of Diagnostic Kits we have already seen the lot of benefits of the diagnostic kits but there are two important benefits that can be derived from the use of diagnostic kits and that is in field decision making process. They help us in In-field decision making process and they reduce the cost per sample of analysis. So they helped us to rapid test in the field within minutes, this then enables the commencement of the management strategies quicker than if a sample was sent off to lab which is an obvious benefit for yield. So it saves time and quick detections means easy and immediate recommendations for management of the problem that is associated in the field, because sending the sample to lab means time is more consumed in the process and to get back the desired result and they are normally cheaper in nature because of no need to send the samples to the lab. Whereas, using a rapid test means the cost per sample can be reduced because, they are specifically designed for this specific pathogens and they can be easily handled by all kinds of personalities that are associated for plant disease diagnosis. So with this we have come to an end of the second week of this course Detection, Diagnosis and Management of plant diseases and in the next week we will be talking about the conventional methods of plant disease diagnosis and for aware of the tools and techniques that has been basically used for plant disease diagnosis. We will be looking into those tools and techniques in a more elaborated way in the following weeks.

Thank you very much.

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PDF: Disease Diagnostic Kits

PART III

WEEK 3: ADVANCED AND MOLECULAR DIAGNOSTIC TECHNIQUES

1 Nucleic Acid Based Techniques

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Transcript

Hello!! I welcome you all to the third week of the course Detection, Diagnosis and Management of Plant Diseases. In the particular course of this week we will be talking about advanced and molecular techniques used in plant disease diagnosis and we'll start with nucleic acid-based techniques.

So we all know that nucleic acid-based technique is very sensitive and very specific and that requires that is why it has been used widely in today's context for detection of plant disease of various origin. Plant pathogens that detects through DNA and RNA are based on overcome the certain diagnostics and pathogen taxonomy that enables a rapid and accurate detection and quantification of plant pathogens. Very important to know that the K. Mullis has received Nobel Prize in 1993 due to his discovery for amplification of nucleic acid sequences using the technology known as polymerase chain reaction. In short we popularly known as PCR. Based on the fidelity of DNA hybridization and replication PCR isused for highly specific detection of fungi, bacteria, viruses and phytoplasma So it's been a very common and popular technology for using in plant disease diagnosis. PCR technique can provide very high sensitivity and specificity due to the fidelity of DNA amplification.

Success of PCR depends on efficacy of DNA extraction and performance is affected by inhibitors present in the sample assay, polymerase activity, PCR buffer and concentration of deoxynucleoside triphosphate. In addition application of PCR for pathogen detection requires designing of a primer to initiate DNA amplification which could limit the practical applicability of this technique for new and unknown pathogens. If the DNA sequence of the new or unknown pathogen is not available then designing primer will not be possible and that is why this is a limitation of PCR. PCR based methods can be used for different genomes like single[1]stranded RNA (ssRNA) single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) and PCR offers several advantages such as the capability to detect a single target in complex mixtures this is highly significant and, rapid and specific detection of multiple targets, and the potential to detect unculturable pathogens, such as viruses and some bacteria and phytoplasma which are not been culturable so far. So this is the strength of the PCR that apart from the known pathogens or culturable microorganisms it can also detect unculturable microorganisms or certain fungi or bacteria or phytoplasma along with viruses.

So Genome extraction of pathogens could be done either following manual techniques or using commercial kits specially designed to extract nucleic acids from different types of plant material. So DNA extraction from the plant material is not a problem nowadays as we have apart from manual techniques we have diagnostic kits or extraction kits they are commercially available for genome extractione. All molecular detection methods for detecting plant pathogens are basically based on accurate design of oligonucleotides and probes. Target sequences can be found in using the Gen Bank Nicleotide Sequence Search program that is NCBI and where most of the genomic data is stored and one can very easily find out the genomic resources from NCBI site. For searching of nucleotide bases normally a tool known as BLAST is used and the program that is BLAST and program is designed for analysis of nucleotides. Specific nucleotide regions are selected and

primers for specific DNA or RNA targets can easily be designed using this blast tool. Primers are designed to pair with unique DNA regions from target organisms for DNA amplification and detection. The presence of amplification product confirms the presence of the organism in the tested sample. The amplified product then visualized through agarose gel electrophoresis using a stain known as ethidium bromide (EtBr). However nowadays less toxic and more sensitive stains like SYBR GREEN is used for detection method using under UV irradiation. Generally, PCR can be performed in two to three hours but advanced systems can deliver results in few minutes as well.

So, briefly PCR reaction mixture is prepared in a PCR tube, it composed of the template DNA, then the primers that complements the target the regions of the template DNA. Then along with free nucleotides, then Taq polymerase for amplification and then the mix buffer, all these are incorporated into the PCR tube at a specific proportion and that PCR is run and the PCR cycles include the first step that is denaturing that is separation of the two DNA strains at high temperature which is at around 95o C followed by temperatures that is set for annealing of PCR primers which is set at around 55 degree Celsius however it depends on the handling temperature of the primers. Then the next step is primer extension and the temperature here set is 72 degree Celsius and this cycle is then completed for next 32 to 35 cycles and after the limited number of cycles it is the PCR product is verified by running through in the agarose gel electrophoresis and the presence of a band that indicates the PCR positive results that confirms the presence of the particular pathogen in that particular genome that is extracted from the plant tissues.

Another method is DNA microarrays. So DNA microarrays are promising high throughput tools and can detect multiple pathogen at the same time. This is the major advantage of DNA microarray. So, microarray consists of a solid matrix usually a glass slide on which oligonucleotide probes or other DNA fragments are placed in very precise locations at high density. So this is a typical example of a

DNA microarray chip where the in that microchip this is the DNA probes that are arranged at specific locations at very high density. 06:46 The target DNA sequence in a sample then hybridized to the probes and detect by fluorescence. So the target DNA sequence is then hybridized with the probe and then it is detected with a fluorescence light. The advantage of microarray based detection is the combined powerful nucleic acid amplification strategies with a massive screening capability resulting in high level of sensitivity, specificity and high throughput capacity. It can detect many different pathogens in a single assay so, that is why DNA microarray is also being used to detect multiple pathogens from a single species.

Another important DNA based technique is known as FISH in short in full it is known as Fluorescence In Situ Hybridization. This technique used 16S or 23S ribosomal(rDNA) oligonucleotide probes labelled with fluorescent dye in combination with fluorescence microscopy. The FISH probes of size (20-30mers) recognized the pathogens in plant tissue cells, fixed in a microscopic slide and hybridized it with target gene in the pathogen in the plant samples. The probe target hybridization can visualized by fluorescent light. FISH was successfully used with probes to target the 23S rDNA to detect Ralstonia solanacearum in potato peels. So this is an another DNA based technology where a fluorescent probe is hybridized to a specific location on the microbial or pathogen genome and then it is visualized under fluorescent or confocal microscopy to see that whether that particular fluorescent probe is visible or not. Visible of fluorescent tag is confirmatory for the presence of that particular genome that is targeted. This could also be used to detect fungi and viruses and other endosymbiotic bacteria that infect plants. The high affinity and specificity of DNA probes provide high single cells sensitivity in fish because the probe will bind to each of the ribosomes in the sample. However the practical limit of detection lies in the range of around 103 CFU/mL. So this level or this limit should be achieved before observation under FISH or under fluorescent microscope. In addition to the detection of culturable

microorganisms that cause plant diseases FISH could also be used to detect yet to be cultured so-called unculturable organisms in order to investigate complex microbial communities. So the probe can be designed to target even those un culturable microbes which are yet to be cultured. Further, FISH can also be used for detection of pathogens in which plants symptoms are yet to be produced. For example, here the downy mildew pathogen in impatiens plant was detected by using the fish technology. So these are the presence of sites of downy mildew in the impatiens leaves and this is again a opportunity to identify pathogen before its production of symptoms.

So, in short we have seen that nucleic acid-based technologies can be very successfully deployed in pathogen diagnosis and detection of even unculturable or yet to be cultured or even nonculturalable entities like viruses could be detected very successfully in presence even before production of the symptoms by the plants and so that it helps the farmers or growers to take adequate control measures at threat or appropriate time before the pathogen cause severe damage to the crop plants.

So, with this we have come to an end of the nucleic acid-based technologies and in the next topic we will be looking into the sort interfering like RNA based technologies.

Thank you very much

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2 siRNA Based Techniques

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Transcript

Hello, welcome back to the second talk of the third week and that is si based RNA based detection technology . RNAi is a basic phenomenon that exist in all living beings, including plant system, where, a foreign RNA is degraded by the inherited small nucleotide of RNAs strengths and there by this mechanism plant defense itself from certain pathogen that invades the plant tissue system, and this particular technology has now been utilized commercially for basically develop resistance in the plants again certain plant pathogens. So what is RNAi silencing or RNA interference – It is a cytoplasmic cell surveillance system to recognize double stranded, RNA and specifically destroys single and double stranded RNA molecules, homologous to the end user using small interfering RNAs normally known as siRNA as a guide. Viruses are both inducers and targets of RNAi that constitutes a fundamental antiviral defense mechanism in eukaryotic organisms. It is particularly important in plants that use RNAi to recover from virus diseases. The use of high throughput sequencing of small RNAs (sRNA) from plants can successfully identify the viruses infecting them, including previously unknown viruses, even in extremely low titre symptoms infectious. So plants high throughput sequencing helped in identification of

those viruses particularly of unknown origin because of this high throughput sequencing of the small RNA fragments that is get sequence along with the plant RNAs. RNA silencing constitutes a fundamental antiviral defense mechanism in plants in which host enzyme cut viral RNA into pieces of 20-24 nucleotides in size. When isolated and sequence and en-mass properly assembled, this virus derives small RNAs sequences can reconstitute genomic sequence, information of the virus being targeted in the plant. So once these high throughput sequencing is done followed by its assembly is done, then it takes out the genomic sequences which is differing from the plant origin, and that is why it is able to now differentiate the genomic sequence of the unknown pathogen particularly the viruses which infect the plants and causes disease.

This approach is independent of the ability to culture or purify the virus and does not require any specific amplification and our enrichment of viral nucleic acids. Using this technique known and novel DNA RNA viruses as well as virus as well as viroids have been identified at sensitivity levels comparable to PCR. So, even presence off a low amount of RNA or DNA genomes of the viruses this technique can be useful to detect of this unknown viruses. In short, the double stranded RNA is broken down or cut into piece smaller pieces of twenty to twenty four nucleotides sizes by a plant enzyme known as dicer, then one strand of the small double stranded RNA is then incorporated into the RISC complex and then, along with the RISC camp, complex this guide RNA goes for surveillance in the plant system and whenever it finds a complimentary sequence or particularly mRNA of a particular gene. Then it goes and bind with that particular side and then, with the help of these, RISC complex, this mRNA is then digested and then further fragmented into smaller pieces. So this is how the RNAi technology works, and if it is the originated from a virus , then this is a technology how this virus can be negated in the plant system. So here the same mechanism is depicted where double stranded RNA virus is then cut down into smaller fragments and then one fragment which serves as a guide RNA it incorporates with

the RISC complex and then it goes and find the new template of the RNA which has complementary sides and then it cuts down into smaller fragments. So this small fragments of the cut virus RNA can be subjected to high throughput sequencing, followed by assembly into contigs and search for similarity in database using the blast can help us to identify the virus off which these fragments belong to or if it doesn't belong to any of the known sequences in the database, it can be identified as a new virus. RNA silencing recognize double stranded, RNA and eliminates RNAs homologues to the inducer RNA, by cleavage using RNase III endonucleases called dicers. Plants encodes several Dicer-like enzymes that recognize and cleave long double stranded RNA (dsRNA) which is off 21-,22-, upto 24-base: pairs, fragments and acts as siRNAs. siRNAs then binds to ribonuclease H-like proteins in the RNA induced silencing complex which is in short known as (RISC) and are used to detect homologous single-stranded RNA (ssRNA) molecules for cleavage, producing more siRNAs. In plants, RNAi becomes amplified when the cleaved RNA recruits and RNA-directed RNA polymerase to generate more double stranded (dsRNA), which is again cleaved by a dicer protein to produce secondary siRNAs, that are once again able to detect and cleave homologous RNA in a type of 'degradative PCR' cycle. This leads to the accumulation of large amounts of siRNAs with homologous to the invading virus. So, this is the basic mechanism how the siRNAs are generated in the plant system which can be even enhanced its copy number with the technology using RNA-directed RNA polymerase and then further accumulation of siRNAs which we call it as secondary siRNAs and this accumulate siRNAs are being now sequenced for identification of the new virus. Application of Next Generation Sequencing (NGS) for siRNA detection. NGS platforms such as illumina (Solexa) and SOLid generates massive amounts but rather short reads of nucleotide sequences. With them a set of bioinformatics tools are used to de novo assembly of such short reads and deep sequencing of siRNAs could thus lead to detection and diagnosis of plant viruses.

So, in this particular case for detection of viral organism which is noble in nature:

The NGS platforms Illumina and SOLiD is being proved to be very helpful. NGS platforms have been used to detect- Reverse Transcribing Viruses, Known Viruses, New Viruses and even mixed infections or Defective RNA/DNAs as well as Contamination. So, NGS platforms have been used for various purposes for correctly identifying and detecting the casual agent of plant symptoms. So, with this we have come to an end of the siRNAs based diagnostic technology. In brief, it deals with particularly the NGS technologies are used for sequencing of siRNAs and this nucleotide sequenced siRNAs can be assembled and then we can get an idea of the entire genome of virus that is causing the plant symptom and if it is subjected to similar sequences in the NCBI database we can identify the existing virus that is being infecting plants and different plant species. Or if the sequences are entirely different from the sequences data being deposited in NCBI database, then it categorized to be a new virus that is causing the plant disease.

So with this we have come to an end of second talk of this week that is siRNAs based diagnosis and in the next topic will be talking about genomic based diagnosis. Till then….

Thank you very much.

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PDF: siRNA Based Techniques

3 Genomics Based Diagnosis

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Transcript

Welcome to the third talk of this week, that is genomics based diagnostic methods. In the previous talk we have already mentioned about the genomics application in detection of and sequencing of SiRNAs. But we will be talking about more detail into the different genomic platforms that is based on high-throughput sequencing.

So genomic based sequencing is also known as NGS or next-generation sequencing technology. It is basically pyrosequencing or high-throughput sequencing, and it is revolutionizing the field of pathogen detection in a variety of plant samples. Unlike other molecular methods which require prior knowledge of sequence information on the pathogens NGS approach is unlimited making it possible to detect any known and novel pathogens in a single assay. So, this is the greatest advantage of this particular genomics assisted diagnosis program, where we do not require any prior knowledge on the microorganism that is actually causing the illness to the plant. So, this approach is not a rapid test but important for identification of unknown bacteria viruses and viroids, has no previous sequence data of the organism is needed. So, that is why it is we mostly use for those pathogens which are not previously reported or goods origin is not certain. No previous sequence data

of organism and there is also no need to culture the organism only we know that so, far we were able to culture only 10% of the total bacteria that is present on this globe and approximately, 90 percent of the total bacteria which are still unculturable in nature. And, that is why we are not aware of those microorganisms in general but we can definitely sequence those microorganisms which are not culturable through this particular technology that is genomics assisted in NGS platforms. Discovery of new viruses/viroids and new hosts has increase very rapidly after the introduction of the NGS. So, application of NGS in diagnosis of plant pathogens let us go for a case study how it is helping new pathogens to be identified.

The advent of high-throughput sequencing has made it feasible to determine the genome sequence of the phytopathogenic bacteria Pseudomonas syringae pv. actinidiae in short we call it (Psa). This is the causal agent of an emerging pathogen that is bacterial canker of kiwifruit in New Zealand. In a very short period of time this particular pathogen started infecting all the kiwifruit orchards in New Zealand some eight to nine years back and it causes a severe alert severe problem to the New Zealand kiwifruit growers as well as in the New Zealand economy that based on these industries. The original outbreak strain was sequenced using a Roche 454 GS junior sequencing platform and within the first few weeks the outbreak of a draft genome sequence was assembled and the causal agent was diagnosed and the biovar was established so it was possible to identify the particular pathogen along with its biovar only through the technology of NGS. All other relevant technologies were able to identify it up to a certain level but certainly not up to the level of biovar and that is why NGS technologies has shown in this particular case that how it is important and how it is giving service to different nations. So proper management schedule could be adopted due to rapid diagnosis of the pathogen in New Zealand and therefore, a fruit industry or Kiwi orchards were saved from the pathogen because of the help of this NGS technology. There are different NGS platforms for example like 454, Illumina, PacBio, Illumina MiSeq and these different platforms provide different type

of services for example 454 platforms gives a 1 GB equivalent per run then in case of Illumina HiSeq 2000 we receive less than 300 GB data per run in case of PacBio it is less than 500 MB per run and it is 8.5 GB/run in case of Illumina MiSeq. But the read lengths of all the platforms are different in 454 the read lengths are less than 900 basis whereas, Illumina it gives short reads of around 100 bases in case of PacBio it gives very long reads of 3,000 and more bases and whereas Illumina MiSeq sequence it also give short reads of around 100 bases. So, depending on the investigating as necessary we can go for different types of sequencing platforms for identification of on diagnosis of the pathogens concerned. This is another chart where it is giving a comparative features of different platforms again HiSeq 2500, HiSeq 2500 with high throughput as well as Rapid mode then MiSeq, PacBio RSII and you can see that read lengths vary drastically in different platforms and that is why their yield per lane is also different incase of different platforms.

NGS can help in identifying of known and unknown plant diseases within a day that is the beauty again of the particular NGS platforms that it takes so less of a time and if it is properly utilized within a single day one can come to an conclusion what type of microorganism or what type of pathogen is associated with the disease that is manifested in a particular crop species. With NGS one can find out within a day whether or not plant material is infected with pathogens.

As this technique captures both usual and unknown suspects it is a true breakthrough in genetic plant diagnostics. PCR technique detects patterns like fungi, viruses and bacteria by multiplying specific parts of their DNA. The disadvantage of this is that the search is highly selectively one base his/her assessment of which pathogen is likely to be present on certain symptoms and then one adapt the analysis accordingly. So in case of PCR one has to presume that probably this pathogen is infecting the plant and one will design primers accordingly to detect that particular pathogen whereas in case of NGS no such assumption is required as its sequence the entire genome whether from the pathogenic fungi bacteria or

virus or from the plant and then it differentiate the genome or assemblies that is not of plant origin or that is not of the host plant so that it can then are compared with the NCBI database and then see that unknown sequences match with a another known pathogen and then it can be very easily identified on the basis of this NCBI search. Whereas, if it is search gives no result then again one can conclude that the sequence that is obtained from the possible pathogen associated with the host plant is of a new or unknown pathogen origin.

So billions of building blocks per sample is generated so, unlike old sequencing techniques, NGS maps billions of genetic sequences for an entire plant sample. One can scan billions of nucleotides, the building blocks of DNA, and the order of this nucleotide determines to which type they belong. So again NGS generates billions of database and this billion building blocks can be then assembled and then they can be searched on NCBI database to come out to a conclusion which is quite different from the other nucleic acid-based technology like PCR technology which search for a definite length site specific amplification of the primers and then it amplifies only a particular gene or segment of a gene for identification of the pathogen origin. This reveals that plant's own sequences and allows us to see which divergent sequences are present in the sample. One can conduct his/her analysis using advanced software that have 'fed' with genetic information on plants and pathogens which enable us to quickly issue a reliable and definitive answer. So depending on the quality of the DNA sample that answer can be even provided within a day. So this is how this NGS platforms are helping.

The speed is a major advantage in a sector where time is money for instance if a quarantine organism is discovered in the shipment normally all other biological products in the shipments are detained. So this leads to a significant loss of value. So a quick conclusion is required and that quick conclusion can be achieved by using of NGS platforms that is, within a day or two were it can be established that whether which seed lots or which biological material is infected and which is which lot is not so, that it can be released from the

shipyard and we can prevent the loss of its value. The same principle applies to cultivation the longer it takes to acquire results that are certain the longer one has to wait to combat the disease. So all parties in the chains right from the producers, importers to exporters they all benefit from the rapid diagnostics because it can give them considerable cost savings. So NGS platforms is helping both at commercial level and different growers and producers and even exporter level so that they are able to reduce their a loss because of probable occurrence of a new pathogen.

So tracking and tracing the NGS technology is also suitable for tracking and tracing. Many bacteria and viruses are transmitted by a plant material like seeds. Because of this material are transported on such a large scale however in some times it is difficult to trace the origin of an infection. So since this is again a difficult situation so NGS platforms can come into rescue in this particular situation as well it ascertains the sequence of a pathogen and that we can figure out information such as this occurs naturally in a country or not so once we are certain that okay this seed lot is infected but the pathogen is also present in the same country then there is no required of quarantine regulations to be taken into account. So this type of decision making process can be achieved by NGS platforms.

So what are the steps involved in NGS based diagnosis – it involves mostly the first step the collection of specimens, then convert conventional testing of the specimens then extraction of DNA and RNA followed by sequencing, and then finally through bioinformatic tools we can go for identification of the pathogen.

So with this we have come to an end of the today's talk where, we have seen that NGS can help us to accurately and very correctly detect the biological of pathogen that is causing a disease in certain crops or that is present in a particular shipment and we can identify the disease-causing agent at even viable level within the shortest possible time. So that is the importance and significance of NGS platforms and that we have also seen what are the different and NGS platforms that are available. Currently, you provide services to

growers and exporters. So in the next talk we'll be talking about immunology and antibody based diagnosis technologies.

Thank you very much.

Download

PDF: Genomics Based Diagnosis

4 Immunology/Antibody Based Techniques

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Transcript

Hello welcome to the fourth talk of this week, that is Immunology and antibody based techniques. In the previous talk, we have talked about the use of NGS platforms and plant disease detection and diagnosis. And here we will be talking about at different method that is Immunology or antibody based detection techniques. So Serological assays for plant pathogens, such as viruses, cannot be cultivated ad hoc and hence serological assays were developed to detect them. So definitely we need to have different technologies for those particular microbes that causes plant diseases like viruses which are not culturable and that is why serological methods is also helpful or useful to detect certain pathogens of that kind. More than a thousand other pathogens, like bacteria and fungi, can now be detected using polyclonal as well as monoclonal antisera and techniques such as ELISA western blots, immunostrip assays, dot-blot immune binding assays, as well as serologically specific electron microscopy (SSEM). So these are all techniques that is serology based, and these techniques are deployed for identification of various pathogenic virus, bacteria and fungi.

Among them, ELISA was first employed, 1970s and till now it is the most popular method used for high throughput detection

of certain plant viruses. The sensitivity of ELISA varies depending on the organism, the sample freshness and a titre, for instance, a bacteria can be detected at 100cfu/mL-1 .Antibodies need to be stored at lower than -20o C, and it should not be defrosted several times because the antibody lose its properties if it is defrosted several times. So this is kind of a drawback of this particular technique. So ELISA in full, it is known as (enzyme linked immunosorbent assay) So ELISA is a molecular method for identification of disease based on antibodies and change in the assay. In this method, the target epitopes- that is the antigens of the pathogen such as from viruses, bacteria and fungi- are made specifically bind with antibodies conjugated to an enzyme. So, this is the major principle behind ELISA technique where the epitope or the antigen is made to bind with antibodies, which is linked with an enzyme.And then, when the substrate is added this enzyme can change color of the solution, but normally, it turns into yellow color The detection can be visualized based on color changes, resulting from the interaction between the substrate and immobilized enzyme. So here this is an example of PCR plate , where you can see that the yellow color wells, giving the positive result for the particular pathogen and the more darker the refers to the more number antigens present in the sample. So the lighter color wells indicate lesser amount of antigens presence in the well. The principle how ELISA works, based on this – There are four different types of ELISA that are designed that is direct ELISA, indirect ELISA, sandwich ELISA as well as competitive ELISA. All this four ELISA techniques are used based on their necessity and based on their applications. So let us talk about Direct ALISA – Where a target protein or a target antibody is immobilized on the surface of microplate wells. Here, the target proteins are immobilized in the microplate wells and it is incubated with an enzyme- labeled antibody, is the enzyme labeled antibody when it is incubated together and then, when the substrate is added, then the color of the solution turns yellow. So this is the process, we call it as direct ELISA and yellow color development indicates positive outcome of

the interaction. Next is indirect ELISA – Where the target protein is immobilized on the surface of micro plate wells and incubated with an antibody to the target protein. We call it as primary antibody. Then if the secondary antibody is added against the primary antibody and after washing the activity of the microplate well bond enzyme is measured.

So here what happens? The protein is immobilized in the microplate well. Then, first primary antibodies are added which is not enzyme linked and followed by in the next step. Secondly, antibody is added, which is linked with an enzyme and then when substrate is added then this enzyme linked antibody with substrate interacts and the color of the solution turns yellow. So this gives again a positive indication of presence of a particular pathogen that is present in the same sample. Although indirect ELISA requires more steps, then Direct ELISA labeled secondary antibodies are commercially available, eliminating the need to level primary antibody each and every time.

So it saves certain amount of cost as primary antibody are not labeled with enzyme and rather the function of enzyme is achieved through use of commercially available secondary antibody. Next, is Sandwich ELISA – The antibody to a target protein is, immobilized on the surface, microplate wells and incubated first with the target protein and then with another target protein specific antibody, which is labeled with an enzyme. After washing, the activity of the microplate well-bound enzyme is measured. So in sandwich ELISA first of all, the antibody is immobilized in the mictoplate well, in contrast to the antigen then antigen is added, at the second stage where primary antibody will then bind with the antigen followed by addition of secondary antibody that is linked with enzyme and this will bind with the antigen and then when substrate is added the color of the solution turns to yellow.

So, it indicates again positive off the particular pathogens present, and this is what we call it as Sandwich ELISA. Since the antigen is present in between two antibodies, so this particular type of ELISA method is known as sandwich ELISA. The immobilized antibody,

that is orange in color and the enzyme labeled antibody which is green in color, must recognize different epitopes of the target protein. So both the antibody's that is the yellow color antibody as well as the green color antibody the both recognizes different epitopes of the target protein, and that is a important criteria for designing of for use of antibodies for sandwich ELISA. Compared to direct ELISA the sandwich ELISA the (combining antibodies to two different epitopes on the same target protein) has a higher specificity and Sandwich ELISA useful for applications that require high accuracy. So this is another method ELISA for those samples which requires high accuracy data generation

The last one is competitive, ELISA here antibodies specific for a target protein is immobilized on the surface of microplate wells and incubated with samples containing a target protein and is known amount of enzyme-labeled target putting.

After the reaction the activity of the microplate well-bound enzyme is measured. So this particular technique is used again, just like a Sandwich ELISA. First, the primary and the antibody is immobilized in the microplate well then addition of the enzyme linked protein that is added and when the substrate is added, then this enzyme linked antibody proteins will turn into yellow color gives a positive indication. So the competitiveness arises, depending on the amount of the actual antigen present in the sample. More the amount of antigen present in the sample, lesser the amount of enzyme-labeled antigen will be present, and that is why the color will be lighter in color in this case, as we have more amount of antigen, presence it will occupy more number of antibody on the less amount of enzyme linked antibody, will be occupied here, and that is why the color of the solution will be lighter yellow. Whereas, in case of lesser amount of antigens presence, more number of antibodies, will be occupied by enzyme linked antibodies, and the color of the solution will be brighter or darker yellow. So this shows the competitive ability, of the antigens, and that indirectly tells us the story whether the amount of antigen that is present in higher quantity or lower quantity in the sample. Next technique is

Immunofluorescence (IF) – So it is fluorescence microscopy-based optical technique, it is used for detection of pathogens infections in plant tissues. For this technique plant samples are fixed in to a microscopic slides in thin tissue sections, then detection is achieved through conjugating, a fluorescent dye to the specific antibody to visualize the distribution of the target molecule throughout the sample. So what it does is that, just like in other microscope we prepare a thin slide of the section of the target sample fixed in a microscope slide and then at fluorescence dye and then observed under fluorescence or confocal microscope. So the specific dye's will go and bind to specific sites and once the dye binds to the target sites, then under fluorescence microscopy , then this fluorescence colour is very observed, and we can then confirm the presence of the particular pathogen or not. So, Immunofluorescence has been used to detect onion crop infection by the fungus, Botrytis cinerea.

The Immunofluorescence has also been combined with other techniques such as FISH for detection of crown rot pathogen Dickeya species in potatoes, which is an emerging disease in Europe. So it has a lot of significance whether Immunofluorescence has been used singly or in combination with the technology of FISH that is Fluorescence in situ hybridization. Even new and emerging pathogens pathogens can be detected in plant samples.

The other method is Flow Cytometry (FCM), so this is a laser-based optical technique, widely used for cell counting and sorting biomarker detection and protein engineering. But this technique can now also be used for detection of plant pathogens whether there is sufficient amount of a particular cell count is available based on it the detection of the pathogen can be achieved. So technically, samples having the pathogen cells is sent through a fluid along with the tube and then it is subject to a narrow tube and during they passes of the sample through the narrow tube the light source is allowed to visualize the samples and, depending on the cell nature, the light get scattered, some of the light get passed and some of the lights get steak scattered and this scattering pattern of the light is then recorded by different electronic devices and based on these

electronic signal, since different pathogens has a different capacity to reflect the lights so, based on this technology, based on this electrical signal that is generated the pathogen is identified in the sample. So, this is again a very new technology and it is still not been very highly used in plant pathogen detection, but still it has a lot of scope for detection of plant pathogens, although it had been very frequently being used in determining the microbial pathogens that is causing food poisoning in certain food samples. So, with this we have come to an end of today's talk that is antibody or immunological based technology for detection of plant pathogens where, we have talked about different techniques of ELISA then , Immunofluorescence and then Flow Cytometry. Next, time we'll be talking about advanced DNA based point of care, diagnostic methods, for detection of plant pathogens.

Thank you very much.

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PDF: Immunology/Antibody Based Techniques

5 Advanced DNA-Based Point-of-Care Diagnostic Methods

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Transcript

Hello, welcome to the last talk of this week. That is DNA-based advanced point of care diagnostic techniques. So point of care techniques are gaining popularity because they don't require sophisticated equipments. They are able to make immediate decisions and they are very easy to handle. So point of care diagnostic essays performed were a very cheaper rate as well and that is why they are also in very high demand. We know that PCR based techniques, although they have several advantages but certain limitations of PCR based technologies is that this technology cannot be taken directly into the fields and it requires electricity and other supporting devices. So it's seriously limits its adequacy for point of care applications. As an alternative, isothermal DNA amplification methods are ideally suited to overcome this limitation of PCR. For instance, Isothermal amplification combined with lateral flow stripes and portable fluorometers have been successfully used for point of care, detection of pathogen DNA. so point of care, DNA extraction methods. In plant tissues DNA extraction method requires ability to efficiently remove in number of chemicals that

can inhibit the DNA amplification reaction. So this is very important, very critical that to have a successful PCR run, we need to have a DNA extracted properly from the plant samples and remove all the chemicals that can inhibit its amplification. So lateral flow device in short, we call it as (LFD) DNA extraction method has been reported as rapid and efficient point of care testing and has been successfully used in plant pathogen detection. So this LFDs are being very useful in point of care detection of plant pathogens because it requires very less amount of substrate as well, less, very less time to get the DNA. This method involves sample disruption in extraction buffer using metal ball bearings before transferring the lysate into the release pad of a LFD nitrocellulose membrane.

The technology is very simple. Take the sample, put it into the sample buffer in the tube that contains two ball-bearings, that is metallic origin and then it crushes the tissues in a way and the tissue fluid is then transferred to the lateral flow device, then nitrocellulose membrane. Then small piece of membrane is excised and added into the DNA amplification reaction such as PCR or other isothermal amplification methods. So the membrane binds the DNA that is present in the tissue and this small portion of the membrane is still transferred to the PCR tubes or isothermal amplification tubes and this serves as a DNA that is present in the sample. The isolated DNA is very stable on the membrane at room temperature, which allows the extraction to be performed in the field condition. So that is why the point of care DNA extraction methods using nitrocellulose membrane has been so popular that it binds the DNA, it does not require any specific temperatures to be maintained and that's how it is being used for point of care detection. Another, method is use of simple cellulose-based dipstick that allows plants sample processing as little as 30 seconds. Plant tissues are here basically macerated by shaking in a tube containing extraction buffer and 1 to 2 ball-bearings for 8to10 seconds. Then cellulose dipstick is inserted in the tube containing the sample before washing it three times in a second tube containing wash buffer, and finally into a tube containing the amplification mix. So

this is again a very simple method, where does the cellulose based dipsticks are immersed, inserted into the plant tissues. And then it is macerated by the help of the extraction buffer and the ball[1]bearings. It is just done for 8 to 10 seconds and then the cellulose dipstick when inserted into the tube, then it adhered the sample and into it and then after washing it in a second tube then that dipstick can indirectly transferred to the PCR or amplification mixer.

So the technology works efficiently in multiple cultivated species including rice, wheat, tomato and sorghum as well as some difficult to isolate plant species like leaves from mature trees of mandarin, lime and lemon. So it can be used to detect pathogen DNA as well as RNA from infected tissues and works with multiple amplification methods such as PCR, LAMP and RPA. So this is another very easy point of care, DNA extraction method using cellulose based dipstick. So application of nucleic acid, isothermal amplification techniques in plant disease detection. So although PCR is highly sensitive and robust, it is constrained by a number of technical limitations. For instance, specificity is highly dependent on the primers used and its inherent sensitivity makes it prone to false positives due to sample cross contamination. So PCR, although it's a very useful and sensitive kit which is able to detect pathogen, which is present in a very low amount, but it has certain limitations and besides PCR also requires electrically powered equipment to perform the thermal cycling, which limits its use of point of care diagnostics. A number of alternative isothermal techniques are now available that can obviate the need of a thermal cycler.

So one of those isothermal technique is Loop-mediated isothermal amplification, in short, it is known as LAMP. So it has widely been used to its high efficiencies, specificity, simplicity and quickness. LAMP requires two long outer primers and two short inner primers that recognize six specific sequences in the target. DNA. The first inner primer containing sense and antisense sequences in the DNA will hybridize the target sequence and initiate DNA synthesis. In the next step, the outer primer carries out the

stranded-displacement DNA synthesis and produces a single stranded DNA which work as a template for the second inner and outer primers producing a DNA molecule with a loop structure. The unremitting cycling, reaction accumulates products with repeated sequences of target DNA of different sizes. The reactant tube is incubated at 63o C to 65o C in a regular laboratory water bath or heat block that helps in maintaining a constant temperature.

So this is one of the greatest advantages of this particular technique. We hear that we don't require different temperature regimes as it is in the case of PCR where we require temperature for denaturation of DNA, then we required temperature for annealing of primer, and then we required a different temperature for extension of primer. But here we require only one temperature that is around 63 to 65 degrees Celsius, which can be maintained in a laboratory water bath or heat block. The amplified product can be detected by naked eyes as a white precipitate or a yellow green color solution after addition of SYBR green to the reaction tube. So here we don't require even the gel electrophoresis or ETM bromide staining for detection of the PCR amplification product. Here the product can be visualized as white precipitate on the tube by even naked eyes or if we add SYBR green, then reaction mixer turns yellow, green in color. So this is a confirmation color change is a confirmation of the positivity of the reaction with the primers. There are three major advantages of this particular tactic. First, it can be carried out at a constant temperature with a short reaction time. This rapid isothermal process makes it ideal for point of care detection of plant pathogens in the field. Since it does not require any electrical support for running the reaction as well as we don't require any gel electrophoresis system to visualize the outcome reaction. And that is why it is a very good tool for use of point of care, detection of plant pathogens. Secondly, it is very high amplification, efficiency and sensitivity as it generates large amount of PCR product with low amounts of input DNA.

And finally, this method is relatively cost effective. As it requires simple equipment to perform the assay. Furthermore, there have

been reports that LAMP generates amplicons with several inverted repeats, which could be potentially used to increase the sensitivity in hybridization assays such as LAMP-ELISA hybridization and LAMP incorporated with colorimetric gold nanoparticle hybridization probes. So formation of the loops is also been considered to be a positive outcome of LAMP reactions because in techniques like LAMP-ELISA hybridization and LAMP incorporated with colorimetric gold nanoparticle hybridization process, it helps a lot. So in brief we can see the reaction procedure of the LAMP technique where this is the template DNA and this is the first forward internal primer that cleaves and in the complimentary site. And then it extends to this direction. There by forming this particular strand and this strand is now used by second primer that is backward internal primer. Then it extends to this directions and lead this synthesis of this new strength. And finally, since this section is complimentary to this, this will come and form a loop and this is complimentary to this region so this will come and form a loop. And this is the second loop it generates. And now this second primer for the second primer, this is exposed and then outer primer is then role comes into play. Then it starts amplifying from this point to the other point and it strands , the particulars in new strain. And then here the second outer primer binds and then it amplifies through this portion till this and it produces the new strength. So this is how the amplification process takes place in case of LAMP technique where two internal primers and two outer primers, they recognizes six sites first, second, then third, fourth, fifth, and sixth. So all these sites are recognized. And this LAMP technique is very useful for detection of plant pathogens under field conditions.

The integration of LAMP with electro chemical sensors offered a robust platform for pathogen detection as it was highly sensitive, detecting as low as 10 copies of the pathogen genomic DNA. So this is again, we can see that it is highly sensitive as only 10 copies of the pathogen, genomic DNA, sufficient for successful electrical chemical sensor of the LAMP process. So LAMP by sensor technology has a strong potential for infill testing, detection and

identification of plant diseases. So we have seen here that various advanced DNA-based point of care diagnostic methods and one among them is the Loop mediated amplification of the DNA. And this technique is so popular nowadays as it requires very unsophisticated equipment, but the outcome of the process is very sensitive and reliable and it does not even require confirmation gel electrophoresis. And that is why this LAMP technology is now rapidly being used by plants disease diagnosticians to detect various plant pathogens and directly at field level. So with this, we have come to an end with a particular point of care technology that is based on DNA identification and we have come to end up the third week as well. In the fourth week we'll be meeting again with the new topics on recent advances in diagnostic technologies. Till then, have a good time.

Thank you very much.

Download

PDF: Advanced DNA-Based Point-of-Care Diagnostic Methods

PART IV

WEEK 4: RECENT ADVANCES IN DIAGNOSTIC TECHNIQUES

1 Biosensors Based Diagnosis

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Transcript

Hello, everybody welcome to the fourth week of the course detection, diagnosis and management of plant diseases. In this particular week we'll be dealing with topics that is 'Advancements in Diagnostic Technologies' and we'll be starting the first talk with 'Biosensors based techniques'.

So, the variety of biosensors have been developed recently and it is been commercialized for various applications and they are helping the diagnosticians to detect plant pathogens in a very efficient and precise. So, depending on the operating principle of the sensor the analytes could be detected that using a sensor based on Electrical, Chemical, Electrochemical, Optical, magnetic or even vibrational signals. So based on this methodologies of biosensing this biosensors are developed for detection of plant pathogens. The limit of detection could be enhanced by the use of nanomaterial matrices as transducers and the specificity could be enhanced by the use of bio recognition elements such as DNA, antibody, enzymes and so on. So biological probes can be of various origins like antibodies, nucleic acids and many other factors like bacteriophages and so on. This all probes can identify the pathogen target and their signal is then transduce through a plug

transduction platform and then the signal is amplified for detection signal in the output devices can help us to actually detect the pathogen that is associated with a plant disease.

So Biosensor platforms based on nanomaterials, nanomaterials display electronic and optical properties and can be synthesized using different types of materials for electronics and sensing applications. The popularity of nanomaterials for sensor development could be attributed to high surface area, high electronic conductivity and plasmonics properties of nanomaterials that enhance the limit of detection. So that is why nanomaterials is gaining popularity for being used as biosensors fordetection of plant pathology. The immobilization of the bio recognition elements such as DNA, antibody in enzyme can be achieved using various approaches including biomolecule adsorption, covalent attachment and capsulation or a sophisticated combination of these methods.

The nanomaterials used for wire sensors construction include metal andmetal oxide nanoparticles, quantum dots, carbon nanomaterials as well as carbon nanotubes and graphene as well as polymeric nanomaterials. so all this includes by development of construction of nanomaterial Biosensors. Nanoparticles have been utilized with other biological materials such as antibody for detecting pathogens like Xanthomonas axonopodis that causes the bacterial spot disease in several plants. Gold nanoparticles based optical immuno sensors have been developed for detection of pathogens like karnal bunt in wheat using Surface Plasmon Resonance in short we call it as (SPR). In addition to single probe sensors nano chips had also been made of micro arrays which contained fluorescent oligo probes that were also developed for detecting single nucleotide change in bacteria and viruses with high sensitivity and specificity based on DNA hybridization. So, varieties of used with of nanoparticles whether it has been used alone or in combination with other enzymes and biomolecules, its application is being white in plant pathology for detection of plant pathogens.

Second group is Affinity Biosensors so compared to the nonspecific nanoparticle based biosensors inclusion of a bio

recognition element can greatly increase the specificity of the sensor. Consequently, the other types of bio sensors have been developed and among them affinity biosensors are very popular. The affinity biosensors the sensing is achieved based on reaction on the bio recognition element and the target analyte. So it's not the basically the particle but it is a particle associated with the bio sensor its affinity for detecting the target analyte, it is important and this bio-recognization basically help the biosensors to detect and then transduce the signal and followed by amplification of the signal. So affinity biosensors can be developed using antibody and DNA as recognition elements.

So antibody based biosensor – the antibody based biosensors provide several advantages such as fast detection, improved sensitivity, real-time analysis and potential for quantification. The biosensors enables the pathogen detection in air, water and seeds with different platforms for greenhouses on-field even post harvest storages of processors and distributors of crops and fruits. The principle of establishing antibody-based immuno sensors lies in the coupling of specific antibody with a transducer which convert the binding element to a signal that can be analyzed such as polyacetylene, polypyrrole or polyaniline. So antibody combined with a nanoparticle that actually recognized the analyte and it is being used very widely nowadays, for detection of certain plant pathogens. So, technically the biosensor is signal distant transfused and then it went for data analysis and finally we get the outcome of the result. Whether the bio- sensor may be antibody based or it may be based on DNA. The other process remains the same and the final output is analyzed based on the data it generates from the biosensor. So antibody based biosensors could detect plant pathogens such as Cowpee mosaic viru, Tobacco mosaic virus, Lettuce mosaic virus Fusarium culmorum, Puccinia striiformis, Phytophthora infestans, orchid viruses and Aspergillus niger and so on. So it has been widely used to detect 0various fungal and viral pathogens from different plant species. Antibody based biosensors technology has made tremendous progress upon implementation

on nanotechnology. Gold nanorods (AuNRs) functionalized by antibodies have been used to detect Cymbidium mosaic virus (CymMV), Odontoglossum ringspot virus (ORSV) for rapiddiagnosis of viral infections. So nanoparticle cube based biosensors has been further enhanced when it is tagged with an antibody. The strength of then anoparticles has even gone beyond itsoriginal limit with the use of this biosensor and this combined biosensor of nanoparticles and antibody gives very high specific recognition of the pathogen analyte and it gives a proper identification of the pathogen that is associated with the plant disease.

Next, is DNA/ RNA based affinity biosensor. Due to possibility of detention at a molecular level the DNA based biosensors enables early detection of diseases before any visual symptoms appear. So this is a very important method of analyzing plant pathogen. There is RNA based affinity biosensor and because it is able to detect pathogens before the pathogen multiplies to a great extent and produce a definite symptom on the host plants. The application of specific DNA sequences have been widely used for detection of bacteria, fungi and genetically modified organisms. Based on specific nucleic acid hybridization of the immobilized DNA probe on the sensor and the underlying DNA sequence, DNA 08:49 based biosensors allows rapid, simple and economical testing of genetic and infectious diseases. The most commonly adopted DNA probe is 08:59 single-stranded DNA on electrodes with electroactive indicators to measure hybridization between the probe DNA and the complimentary DNA analyte. So, once the probe DNA and the complimentary DNA analyte they interacts or hybridized, an electrical signal is generated and based on this signal generation the presence of the bacteria can be called Or the pathogen can be detected on the Device. Bacterial pathogens are detectable by DNA based biosensors due to their unique nucleic acid sequence which can be specifically hybridized with complementary DNA probe. The recognition of analyte DNA is dependent upon the formation of stable hydrogen bond between the DNA probe and the analyte DNA sequence. This is different from the antibody based biosensors

where hydrophobic ionic and hydrogen bonds play a role in stabilization of the 0 antigen-antibody complex. But in case of the DNA based biosensors it is the stable hydrogen bond that plays into a Role. In addition to DNA- DNA hybridization for bacterial detection the specific habitation of DNA and complementary RNA was also exploited for detection of plant viruses such as cymbidium mosaic virus as well as Odontoglossum ringspot virus.

So it is not only DNA and DNA hybridization, it is DNA and complementary RNA based hybridization. That is technology is also being used for detection of certain plant viruses. So, here we have we have seen that how biosensors are being used for detection of plant pathogens. In the beginning we have talked about the nano particles which are used as a biosensors for detection of plant pathogens, then we have also talked about the nano particles which was then located with a antibody with enhanced its efficacy to detect a certain plant pathogens, then we have also talked about DNA- DNA hybridization and DNA/ RNA complementary hybridization methods for detection of certain plant pathogens. And these particular molecules has been used as biosensors for detection of specific plant pathogens. Sometimes even it produces its definite symptom on the host plants the pathogen can be detected on the basis of DNA- DNA hybridization method.

So with this we have come to an end of the first talk of this week and in the next talk we will be discussing about Volatile organic compounds as biosensors for pathogen diagnosis.

Thank you very much.

Download

PDF: Biosensors Based Diagnosis

2 Volatile Organic Compound (VOC) based Diagnosis

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Transcript

Hello!! Welcome back to the second talk of this week, and today we'll be talking about Volatile Organic Compounds (VOC) based diagnosis of plant pathogens. Volatile organic compounds are released by plants and the pattern of or the profile of volatile organic compounds get changed when the plant interacts with its surrounding, or its atmosphere in a comfortable situation or with a stress conditions. So, that is the principle behind the pattern of different volatile organic compounds being released or emitted by the plants into its surrounding.

So, volatile organic compounds emitted from leaf surfaces are terminal metabolites of the host plant and can indicate its physiological health status. Volatile organic profiling may describe 'plant-to-plant' and 'plant-to- pest communication and therefore it's gaining importance. VOC markers like Hexenols, hexenals, hexanyl esters and classes of terpenoids and indoles may help in rapid discrimination of fungal infection and insect vector feeding. Because the pattern of emission of this particular compounds get changed and it follows a definite pattern when it is infected by a

fungal pathogen and when it is being feeded by a Herbivory Insect. So based on this whether the plant is being challenged by an insect or certain fungal pathogens then release of all VOCs get varied.

So applications of VOC profiling and there are certain success stories. Let us go into it. There was a classical example of peanut plants that is Arachis hypogaea were the volatile organic compound profile of a healthy control was significantly different than those of infected with the white mold that is Sclerotium rolfsii. The major differences were in concentrations of methyl salicylate and 3-octanone so the concentration of methyl salicylate and 3-octanone were very high in case of the infected plants with the white mold pathogen and it was comparatively much lower in the healthy control plants.

Then the feeding behavior of beet armyworms (BAW) on healthy and white mold-infected leaves showed that that beet armyworms preferentially consumed more on the white mold infected leaves. That means higher the concentration of these molecules in the infected leaves it attracted the insect that is beet armyworm to the plant but BAW that is Beet armyworm naturally emitted certain trace amount of hexenyl acetate, linalool, and methyl salicylate, which further retarded the growth of sclerotium rolfsii. So this is just a reverse situation that following white mold infection it invited the bet armyworm pest to attack the peanut plants but at the same time once the beet armyworm attacked the peanut plants it started repelling the white mold pathogen from the host plant. So in contrast the emission of induced volatiles of sclerotium rolf'sii infected plants specifically methyl salicylate and 3- octanone, attracted insect vectors such as beet armyworm. Further, VOCs profiling of potato tubers inoculated with the late blight Pathogen (Phytophthora infestans) and Dry rot pathogen (Fusarium coeruleum) after harvest identified 52 different volatiles.And the most abundant compounds were benzothiazole, 2-ethyl-1-hexanol, hexanal, 2-methyl propanoic acid and so on. The differences in the emitted VOCs profile of potato tubers inoculated with Phytophthora

and Fusarium were evident and provide an early warning VOC system for postharvest disease management in potato.

So even if we don't go for detection of the plant pathogen directly by simply going for VOC profiling one can have an idea whether the potato tubers were infected with a Phytophthora or Fusarium, and if it is infected then definitely one can go for adoption of post-harvest management technologies for protecting the potato tubers from Phytophthora and Fusarium. Similarly infection by Phytophthora cactorum that causes crown rot disease in strawberries results in release of p-ethylguaiacol and p-ethylphenol as characteristic VOCs from the infected portion of the strawberry plant Fruit. So this is another signature that the strawberry fruits are infected by the Phytophthora species because it is emitting this particular volatile organic compound and corrective measures or control measures can adopted or applied immediately if these molecules are detected.

Similarly, Cucumber mosaic virus (CMV) in cultivated squash plants. The CMB infected plants showed an overall net increase in quantities of VOCs like Hexenal, methyl, hepten and so many others In all the plants but no major qualitative difference in VOC profile could be identified in infected plants. Both insect vectors, Aphis gossypii and Myzus persicae, were preferentially attracted to CMV- infected plants, in a similar way with beet armyworm (BAW) to mouldy peanut plants. Despite the smaller size and inferior quality of CMV-infected plants. So, this is another example were Aphis gossypii and Myzus persicae these insects were attracted towards the Cucumber mosaic virus (CMV) plants when it was infected with the cucumber mosaic virus (CMV). This demonstrate that the plant is inducing altered VOC profile in response to viral infection and this type of mechanism is popularly known as super normal stimulus. So this is super normal stimulus because the prior pathogen is infecting the host plants and modifying the VOC profiling and this modified VOC profiling is attracting certain insects and which is not a normal phenomenon and that is why it is 06:54 known as super normal stimulus.

The volatile signature of plants could be analyzed using gas-

chromatography technique to analyze the presence of specific VOC that is indicative of a particular disease. To enhance the performance of compounds separation and analysis the gas chromatography is often combined with mass spectrometry to identify unknown compounds in the volatile sample. So this is normally deployed for edification of known compounds but when it is combined with mass spectrometry then even the unknown compounds can be identified in the VOC profile. GC/GC-MS can provide more accurate information about the plant disease due to its high specificity. It also allows the detection of diseases at different stages based on quantitative information collected from the VOC sample.

So gas chromatography coupled with mass spectrometry is a very good system to accurately profile the VOC of healthy and infected plants and based on these patterns one can very well identify the health status of the plant whether it is infected or it is not infected by any particular pathogen and then based on this signature molecules one can start protecting the plants through other corrective measures or protective measures if the profile indicates presence of a pathogen.

So with this we have seen that how Volatile Organic Compounds can help in diagnosis and detection of certain plant pathogens indirectly because if these pathogens are associated then definitely the VOC profile will be of a particular type in comparison to a healthy one. So that helps us to judge whether the seed lot or the plants are whether infected by any pathogen or not. And if it is infected we can very well take either appropriate control measures for protecting the seed lot or the plants from the suspected pathogen.

So with this we have come to an end of this today's topic and in the next talk we will be talking about micro needle based diagnosis which is again a new dimension of plant disease diagnosis using very easy and rapid methods.

Thank you very much.

Download

PDF: Volatile Organic Compound (VOC) based diagnosis

3 Microneedle Based Diagnosis

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Transcript

Hello!! welcome to the third talk of this week and that is Microneedle based diagnosis. We have already discussed about nucleic acid-based technologies which we know that it is very specific and very important but in field diagnosis nucleic acid-based technologies has challenges and to overcome these challenges there is constant improvement of this particular technology where it is being simplified and modified and one among this technology is use of micro needle for DNA extraction for direction of plant pathogens from infected plants under field condition. So in field molecular diagnosis of plant diseases via nucleic acid amplification (NAA) is currently limited by cumbersome protocols for extracting and isolating pathogen DNA from plant samples. To address this challenge a rapid plant extraction method has been developed using a disposable polymeric Microneedle (MN) patch. By applying microneedle patch on plant leaves amplification assay-ready DNA can be extracted within a minute from different plant species. Microneedle extracted DNA has been used for direct polymerase chain reaction amplification of plant plastic DNA without purification. Further, more using this patch device extraction of plant pathogen DNA for example from Phytophthora infestans from

both laboratory inoculated and field-infected leaf samples were performed for detection of late blight diseases in tomato.

So microneedle extraction achieved hundred-percent detection rate of late blight infections for samples after 3 days of inoculation when compared to the conventional Cetyl trimethyl ammonium bromide that is (CTAB) method based DNA extraction method as gold standard and 100% rejection rate for all blind field samples tested. So the microneeddle based extraction method is comparable with most common and most preferred DNA extraction method that is CTAB method and that is why microneedle based DNA extraction method is having tremendous potentiality to be used under field conditions in coming years. This simple cell lysis[1]free and purification-free DNA extraction method could be transformative approach to facilitate rapid sample preparation for molecular diagnosis of various plant diseases directly in the field. The microneedle patch is postage stamp-sized it can directly be placed in a leaf and pressed if we see the microneedle patch then it comprises of small cone like projections which is basically very inexpensive polymers and this postage size stamp of microneedle patch is very convenient to use and this cone like structures of polymers are hardly .8 millimeters long. So when this patch is fixed or pressed against a leaf it then penetrates the leaf epidermis and the DNA is get adhered to the polymer from the healthy plant tissues of Mesophyll cells as well as infected tissues of Mesophyll cells comprising of pathogen DNA as well. So the DNA gets bind with the polymer of the microneedle patch and this patch can now be taken for PCR amplification. That microneedle method has advantages over CTAB method because DNA extraction by CTAB method requires a lot of equipment and it takes several hours. Further, CTAB extraction is a multi-step process involving tissue grinding to organic solvents and centrifuges. By contrast the new micro needle based DNA extraction technique involves only few steps and require only a microneedle patch and an aqueous buffer solution and it can extract DNA within few seconds or minutes. So this is a brief outline of the CTAB method which involves several

processes and that is why it is very time-consuming and usually it takes three to four hours for extraction of DNA.

In comparison to the CTAB method microneedle patch can extract DNA within a minute. So for a farmer or a researcher can directly apply the microneedle patch to the plant they suspect to be diseased, then hold the patch in place for a few seconds and then peel it off. The patch is then rinsed with the buffer solution washing genetic material of the micro needles and into a sterile container. The entire process takes just about a minute. The micro needle techniques purity levels were comparable to other validated laboratory methods of DNA extraction, we have already talked about it and the most importantly the slight difference in purity levels between the micro needle and CTAB samples did not interfere with the ability to accurately test the samples by a PCR or LAMP assay. So although the quality of DNA may be compromised to some extent but it is not having any effect on PCR amplification or LAMP assay process and that is why microneedle based DNA extraction method is very much recommended nowadays for extraction of DNA directly from field Samples. Since DNA extraction has been considered as a significant hurdle to the development of on-site testing tools, the micro needle based technology has now given a solution to the problem. It is an integrated low cost field portable device that can perform every step of the process from taking sample to identifying the pathogen and reporting the results of an assay.

Further, Lateral Flow Device (LFD) could be used to accurately detect the pathogen DNA extracted through the micro needle technology. So both micro needle technology along with the little flow device can be used under field conditions so the problem regarding extraction of DNA is solved through micro needle based technology and the PCR based technology is replaced with little flow devices and that is how in-field the pathogen can be directly detected by using these novelapplications like micro needle based and patch as well as Lateral Flow Devices (LFD). So, we have seen the utility of microneedle based technology for extraction of plant

DNA from the field samples and it is highly comparable to the gold standard CTAB method and it gives nearly hundred percent results from the 0extracted DNA that was used through microneedle patch. So with this we have 0 seen another novel technology of DNA extraction and this particular technologies need to be further popularized and need to be used in field conditions for saving time and resources and for quick identification of plant pathogens that is causing certain diseases under field conditions. so thank you very much in the next talk we'll be talking about artificial intelligence in disease diagnosis.

Thanks.

Download

PDF: Microneedle Based Diagnosis

4 Artificial Intelligence (AI) based Diagnosis

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Transcript

Hello!! welcome to the fourth talk of the week that is, Artificial Intelligence (AI) based diagnosis. Plant disease diagnosis has been traditionally been done by visual observation where involvement of experts is an essential criteria and it is always a expensive matter because one has to travel to the field and at certain times finding an important expert is also a difficult for growers in certain regions. So bringing an expert from a far of place it again a time-consuming and expensive process so artificial intelligence is coming up in a way that it is helping to solve certain issues like those. Due to which consulting experts is not a mandatory incurrent context because artificial intelligence is trying to replace some of the human activities through use of certain machine and machine learning processes. The use of technology to replace human activities and guarantee efficiency is known as artificial intelligence. But the question arises can artificial intelligence help improve agricultural productivity?

So we will just see into it what are the technologies in this field has been gaining and how it is helping the growers to identify

and detect certain pathogens. So artificial application in agriculture is broadly being used for in terms of (i) agricultural robotics, (ii) soil and crop monitoring and (iii) predictive analytics. Farmers are increasingly using sensors and soil sampling to gather data and this data is stored in farm management systems that allows better processing and analysis. The availability of this data and other related data is paving away to deploy artificial intelligence in agriculture. As a result in number of tech companies is investing in algorithms that are being useful in agriculture. For example image recognition is used in potatoes by AgVoice developed by a Georgia based startup for using natural language toolkit for field notes and yield prediction algorithm based on satellite imagery. Various resources developed several artificial intelligent devices that can identify diseases in plants. For example Tensor Flow is a technique known for transfer learning to teach the artificial intelligence to recognize crop diseases and pests damage. It uses Google's open source library to build a library of around two thousand 2,756 AI images of cassava leaves from plants in Tanzania. The success was that the AI was able to identify the disease with 98% accuracy. So this shows the effectiveness of artificial intelligence to being deployed in agriculture for detection 0and diagnosis of plant pathogens.

Another technique is using of image segmentation and soft computing technique to detect plant diseases. In image segmentation the process basically is of separating our grouping an image into different parts These parts normally corresponds to something that humans can easily separate and view as individual objects. The segmentation process is based on various features found in the image. This might be color information, boundaries or segments of an image. So this is the process how the image segmentation technique offers first – Image Acquisition takes place then, Image pre-processing is required then, Image Segmentation is done, then Features Extraction in image is done and finally Detection and Classification of plant diseases are obtained. So, these are some of the examples how this technology works. So this

is an input and this is the output after image segmentation and based on this input and output images the AI detects the particular disease that is occurring on the plant leaf. Here it is banana leaf disease it is caused by scorch disease. Then the other example is beans leaf which is basically infected by a bacterial leaf spot so, this is the input image and this is the output image and, output image is obtained after the segmentation process is done internally. Similarly, it is used for identification of other diseases such as in rose leaf caused by bacterial leaf spot then other fungal diseases in bean leaf and so on.

iPathology: it is another AI based application that is robotic applications and management of plant and plant diseases in agriculture. So robots application can help in precision plant protection technologies. So, intelligence technologies using machine vision or learning have been developed for plant disease detection and identification. A recognition method based on visible spectrum image processing to detect symptoms of religious like citrus greening which is also named as Huanglongbing (HLB) caused by Candidatus Liberibacter species on citrus leaves. The experimental results showed that detection accuracy is as high as 91.93%. This is again a significant improvement where AI based diagnosis is able to detect more than nine times out of ten times accurately. The huanglongbing detection system can detect the pathogen at pre-symptomatic stage this is again a very significant application of AI based technologies because pre-symptomatic stage is normally not able to traced by human eyes. Similarly, Citrus canker caused by (Xanthomonas axonopodis). It causes foliar symptoms and analyzed to evaluate the efficacy of image analysis. The image analysis was more accurate than visual raters for various symptom types. So robots were also used for image recognition process in case of citrus canker and the symptoms were more accurately diagnosed in comparison to the human eye. Then there are certain mobile apps like for example Plantix which is being widely used in several countries of the world and this is a simple app where even laymen can use for detection and diagnosis of a

particular plant disease problem and then and get the information regarding how to manage this problem.

Plantix is a free mobile application which offers farmers and gardeners the possibility to receive decision support directly on their smart phone. Due to image recognition the app is able to identify plant type as well as appearance of a possible disease, pest or nutrient deficiency in the plant. The app can be used very easily by the users where the users have to simply take the image of the infected plant leaf and upload it to the app and then the app recognizes the leaf damage pattern and based on it, it gives an output in terms of information about the probable disease-causing agent and we also provide recommendations for taking adequate control measures for management of that particular problem associated with the plant. So it's a very simple tool based on artificial intelligence being developed and it can be used by all growers and gardeners which may not have very scientific understanding of the disease problem. So, this is how artificial intelligence is coming in a big way in agriculture system. Particularly it is helping plant disease diagnosis and helping us to understand the causal agent of the plant diseases. So, with this we have come to an end of this today's talk where we have seen how artificial intelligence is helping us to detect and diagnose a plant disease. In the next talk we will be talking about detection and 0diagnostic challenges for detection of emerging pathogens. So we will see how diagnostics are helping to detect emerging pathogens in various parts of the world. Till then have a good time.

Thank you very much.

Download

PDF: Artificial Intelligence (AI) based Diagnosis

5 Diagnostic Challenges for the Detection of Emerging Pathogens

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Transcript

Hello!! Welcome to the last talk of the week that is Diagnostic challenges for the detection of emerging practices. We all know that it is very hard to diagnose a new problem when it emerges in a new location, because lot of data associated with it is not available by the time and because of those it is very hard for the investigators to establish the causal agent of a particular disease which is considered to be a new or emerging problem in a new locality. Let us see the example of Pseudomonas syringae pv. actinidae which is the causal agent of bacterial canker of kiwifruit and it was an emerging pathogen in New Zealand somewhere in 2010. By the time of its outbreak the kiwi fruit orchards were not seen this particular type of disease earlier. And therefore there investigators were not able to associate it the problem and the cause associated with it and it was a big challenge for the investigators to establish the causal agent because it is only then corrective control measures can be adopted and lost due to this pathogen outbreak could be checked in a shorter period of time. Little had been known or published about the Diagnostics ecology

and epidemiology of this problem prior to 2010 in New Zealand so it was a challenge for the diagnosticians to diagnose the disease and stops its spreading. An introduction of Psa into New Zealand underscored a number of challenges in the use of molecular diagnostics to detect and characterize the exotic pathogenic bacteria. Normally, what is preferred for use of our application of molecular diagnostics is that the epidemiology of the pathogen is well understood and reliable and specific diagnostic assays are available for that particular pathogen. But these were not available in case of Psa outbreak in New Zealand. So the time of outbreak there was significant knowledge gaps in the biology of PSA and a lack of validated diagnostic tools for the high throughput detection of Psa in kiwifruit plant material was also not available. The timeframe to validate the identification of Psa was further hampered by the need of culture of the causal agent and to conduct pathogenicity test to demonstrate Koch postulates. All these steps normally takes significant amount of time and it was not very preferred at that time to have that much time to establish that particular causal agent. 0o methods for identifications of Psa 0adopted at the time were definitely diagnosis for combination of molecular, biological, chemical and pathogenicity tests and colonies on nutrient agarplates showed little evident that they are of Pseudomonas origin. Like rounded, convex, glittering, translucent and creamy white colonies. But they lacked a fluorescent pigment on King's B medium which again baffled the investigators to establish the actual causal agent. Psa proved to be gram-negative and exhibited characteristics of Pseudomonas syringe on the LOPAT group a test. But by the time of Psa outbreak in New Zealand, PCR assays to identify Psa had been developed however the primers used for PSA diagnosis was not PSA specific as in some situations the primers used to amplify DNA from other Pseudomonas found on the Kiwi fruit as well. So it was only sequencing that was helpful to distinguish PSA from other leaf spotting Pseudomonas on kiwifruit. So these were certain challenges that existed during the outbreak of Psa in New Zealand. Further large-scale testing of Psa requires

DNA extraction from PCR testing protocols or detection in large quantities of samples which was not established during the New Zealand outbreak. So this required an immediate evaluation of DNA extraction methods and PCR techniques for high-throughput detection of kiwi fruit plant material.

PHEL that is the Public Health and Environment laboratory developed rapidly a SYBR Green qPCR assay using the primers Psa F 1 and R 2 to detect Psa in leaf and pollen tissue that the use of DNA-binding dye SYBR green for the detection of PCR amplifications allowed rapid conversion of conventional q peas to qPCR and enable the high-throughput detection of Psa in response setting. So lot of work had been to be done in a very short period of time to establish or to save time and to correctly identify the Psa strains that are present in different plant tissues.

Further Genetic Diversity of Psa was again as challenge because all Psa strains isolated from the earlier Italian outbreaks shared the same repetitive PCR fingerprint and MLST profile but were different to the strains previously isolated in Japan. So this was again a confusion that existed at the time that the profiles generated from the Italian strain is different from the Japan strain. So none of the strains from the Italian outbreaks presents a possessed genes coding for phytotoxins phaseolotoxin or coronatine, further differentiating these strains for those in Japan, Italy and Korea. So analysis of the cts gene of these strains consistently detected two haplotypes that differed by two base pairs Psa strains from Japan, Korea and the Italian 1994 outbreak belong to one haplotype whereas Italian strains isolated from the epidemics in 2008-2009 belong to another haplotype. So this tools has been able to differentiate that the Japan,Korea and Italian earlier outbreak strain has been put into one group whereas, the new strain of Italian Psa outbreak has been grouped into the different haplotype. These studies concluded that epidemics in Italy during 2008 and 2009 appear to have been caused by different Psa population then those previously recorded in Japan, South Korea and earlier Italian outbreaks.

Molecular Characterization of the Psa: that causes the New Zealand outbreak the identification of Psa was the causal agent of the kiwifruit disease was quickly obtained during the response investigation. However the identification of the outbreak strain was more challenging. Although, the pathogen is established but looking into the genetic diversity of the pathogen existed throughout the world it was very difficult to categorize which strain that is causing the outbreak in New Zealand whether it falls into the Japan haplotye or it is fall into the new Italian outbreak haplotype.

Genetic differences have been detected among overseas PSA strains and the question was raised whether the New Zealand strain was similar to those strains causing an epidemic of bacterial canker in Italy.

So the role of NGS came into play in establishing that fact and through application of NGS technology the strainer type was established for the Psa that was responsible for New Zealand Psa outbreak. The original outbreak strain was sequence using Roche 454 GS Junior sequencing platform within the first weeks of the outbreak and the draft genome sequence was assembled within three days. It did allow an analysis of effector and toxin genes known to be very key virulence determinants in Pseudomonas syringe complex. This analysis of drafts genome sequence quickly validated the identification of the New Zealand Psa isolate and provided significant insight into possible strain type. The draft sequence enabled key effector and phytotoxic genes to be screened and revealed some commonalities with New Zealand Psa strain and the more aggressive Italian strain.

So with the use of NGS platforms finally the investigators were able to establish a relationship between the New Zealand outbreak PSA with the modern Italian outbreak PSA strain to be have some similar characteristics. So this is how technologies help to identify or detect the emerging pathogens in a new location and with these technologies we can now go up to identification of the strain in level of the pathogen that is causing the particular damage to the crops associated with and finally we can then practice the

recommendations that is required for management of that particular strain of the particular pathogen.

So with this we have come to an end of the week poll and in the week 5 we'll be talking about special applications of plant disease diagnostics where we will be talking about recent developments and some special applications that are being deployed for plant disease diagnosis. Till then have a good time.

Thank you very much.

Download

PDF: Diagnostic Challenges for the Detection of Emerging Pathogens

PART V

WEEK 5: SPECIAL APPLICATIONS OF PLANT DISEASE DIAGNOSIS

1 Proximal-sensing of Plant Diseases

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Transcript

Hello! Welcome to the 5th week of the course "Detection, Diagnosis and Management of Plant Diseases". In this week in the first talk will be talking about 'Proximal sensing of plant diseases'. We all that in the fields situation its the human eye basically who first try to identify any problem associated with the plants particularly, if the plants are being infected by the pathogen or not. But, although it is a common practice this way of diagnosis may be not be full proof as human eye cannot trace the initial infection of plant pathogens and a human eye only identify if a visible change on the plant or plant parts is occurred due to development of symptoms by the particular pathogen that is infecting the plant. So technologies are coming in a big way to help and identify those pre systematic symptoms in the plants so that the disease can be properly diagnosed and proper controlled measure could be adopted.

The common method for diagnosis of plant disease include visual examination, microscopic evaluation, as well as molecular, serological, and microbiological techniques. But the new sensor-based methods assess the optical properties of plants within different regions of the electromagnetic spectrum and are able to utilize information beyond the visible range. They enable the

detection of early changes in plant physiology due to biotic stresses, which is a limiting factor for human eye and that is why we normally have to wait till the symptom production stage for management of the particular pathogen.

Currently, the most promising techniques are sensors that measure reflectance, temperature, or fluorescence of the leave. Remote sensing is a method used to obtain information from plants or crops without direct contact or invasive manipulation. The concept has been recently enlarged by proximal, close-range or small-scale sensing of plant material. Proximal sensors may be hand-held, machine-mounted or attached to suitable unmanned aerial vehicles (UAVs).

Proximal Sensing vs. Remote Sensing

So, this a comparative between Proximal and Remote Sensing tools. In case of Proximal sensing normally autonomous systems are that can go very close the plants are used which are ground based as well as certain machine mountain cameras and another tools being used then sometimes even unmanned vehicles can also be used for Proximal sensing but mostly these are used as remote sensing tools. Further, aircraft or satellite are used for remote sensing detection of plant diseases.

Various sensors, like sensors like RGB, Multispectral, Hyperspectral, Thermal, chlorophyll fluorescence and 3D sensors are used for detection of plants symptom at various level at Cell, Leaf, Plant, Plot, Field or even at the Ecosystem level. So depending on the uses of the equipment or depending on the requirement this different sensors are used to detect and diagnose plant diseases.

Systems for Proximal Disease Sensing – First one is Thermography. The thermal imaging is a non-contact technique to determine the temperature distribution of any object in a short period of time. Infrared radiation emitted from plant surfaces may be recorded by detectors sensitive to radiation in the infrared region from 8 to 12 micrometer. Each pixel of image is related to a temperature value of the objects surface and may be illustrated in false color image. The technology can be used from microscope

applications to ground-based equipment covering a range from leaf tissues to crop canopies. it is used to detect pathogens like tobacco plants infected with tobacco mosaic virus, sugar beet infected by Cercospora, downy mildew of cucumber caused by Pseudoperonospora, grapevine leaves infected with Plasmoparaviticola, and for apple leave infected by Venturia inaequalis. So we can see that a large number of applications has been used for proximal sensing of certain diseases again seasonal to perennial crop plants.

So, this is an example how Thermographic detection takes place. So this is Fusarium head blight in wheat and this is an infected wheat ear head and this is a healthy ear head and one can see that the thermal reflection or thermography of both infected and healthy shows variations in the different temperature scoring.

Then the next one is Fluorescence Measurements: So, various fluorescence parameters of plants irradiated with ambient excitation light may be recorded for the assessment of photosynthetic activity and the content of chlorophyll and other plant metabolites, e.g. phenols. These methods are very sensitive to detect changes in photosynthesis. Since disease development also affects the crops photosynthetic apparatus like pigments, electron transport chain, enzymes of the CO2 fixing Calvin cycle and the intensity as well as the spectrum of chlorophyll fluorescence are modified in diseased plants, sometimes even before visible symptoms appear. So, fluorescence measurement is again useful tool to detect if the plant leaf or plant is affected by certain pathogens because it causes change in the photosynthesis apparatus. This is an example, the left one is a normal colour image of the plant leaf whereas, the right one is a chlorophyll fluorescence parameter of an apple leaf infected by Venturia inaequalis. So, one can see that the image based on fluorescence parameters are very different from the normal visible range of light and this is how one can see that whether there is any abnormality in the leaf is taking place because of change in the fluorescence pattern.

Then the next one is Spectral Techniques – The reflectance of

incoming electromagnetic radiation in the visible, near infrared and short wave infrared depends on multiple interactions like back scattering at the leaf surface and internal cellular structures, radiant energy absorption induced by leaf chemistry, for e.g. content of pigments, leaf water, proteins or carbon. So, all these factors determine how much light will be emitted back through the electromagnetic radiation. The detection of diseased plants, i.e. plants with a spectrum different from that of healthy ones, using spectroscopic techniques has been successfully used for the blast pathogen of rice i.e. Magnaporthe grisea on rice, Phytophthora infestans on tomato, Venturia inaequalis on apple trees, canker lesions on citrus fruits, Blumeria graminis i.e. the pordery mildew fungi on barley, and Rhizoctonia root and crown rot of sugar beet. So, infections of sugar beet by various leaf pathogens could be detected even pre-symptomatically using this technique.

So, these techniques are useful that is why even if the symptoms are not visible by naked eye in the plant hole but they will change in chlorophyll content or there is change in other physiological or molecular markers in the leaf. This technique can have different ability to detect those affected and healthy plants and that's how they can give us an indication how what are the status of the plants health. So, here we can see that it's wheat leaf infected by rust and you can see the spectrum or the hyperspectral data that is obtained from the infected leaf, it varies depending on the amount of leaf area covered by the pustules of the rust pathogen. So the Spectral Signature of Disease Symptoms can be similar for a particular disease and it varies from disease to disease. So, in case of healthy plants the spectral range is this whereas, the chlorotic tissues have the spectral range of the, there's a middle line, it shows the chlorotic tissue from the rust pustules is developed that is the lowest or third line. So, with this type of spectrum we can very easily distinguish that whether the leaf is affected, whether there is any chlorotic area, whether there is any pustule present in the leaf surface. So, by looking at the data one can very well recognize whether the plants are healthy or infected.

So , with this we have seen how Proximal sensing can be done and what are the tools that are been used, how Thermography or Spectral data can help us in identifying or recognizing an infected plant by using Proximal sensing devices. So in the next class or in the next talk we will be talking about the remote sensing technologies that are used for plant disease diagnosis. Till then, have a good time.

Thank you very much.

Download

PDF: Proximal-sensing of Plant Diseases

2 Remote-sensing of Plant Diseases

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Transcript

Hello! Welcome to the second talk of this week that is Remote sensing of plant diseases. In the previous talk we discussed about the Proximal sensing of plant diseases and in today's talk we will be talking on Remote sensing of plant diseases, that means tools and devices used to sense plat that is being suffered by a particular disease form a very distant place without coming directly in contact with the plant canopy or plant tissues.

So, Remote Sensing is method used to obtain information from plants or crops without direct contact or invasive manipulation. To obtain information on an object by measuring the electromagnetic energy reflected/backscattered or emitted by the surface of the earth. The measurements are possessed and analyzed to retrieve information on the object observed for example plant health in this case. SO, remote sensing is an indirect assessment technique which is able to monitor vegetation conditions from distance, and evaluate the spatial extent and patterns of vegetation characteristics and plant health, in this application. So a plant which is in stressed conditions that is (induced by a disease) reacts with potassium mechanisms that lead to suboptimal growth which show up as changes in variables such as leaf area index (LAI), chlorophyll

content, or surface temperature and thus producing a spectral signature different from the signature of healthy or unstressed vegetation.

So, this is the basic principle how remote sensing works. The spectrum that is produced by a healthy plant and an infected plant based on the lot of chlorophyll or leaf area index or the surface temperature in general so at certain times it is not possible for even a human eyes to go beyond a particular area to see the extent of damage that has been taking place in large scale cultivations.

When plants are exposed to pathogens they activate defense responses whose molecular mechanisms are very complex. At the early stages, when visual symptoms such as lesions on the leaf surface are not present, plants react to those presences of a pathogen with physiological mechanism such as reduction of the photosynthesis rate, which induces an increase of fluorescence and heat emission from the infected plant leaves or plant tissues. The presence of stress factors changes the thermal properties of plants, which in term influences the radiation emitted in the TIR domain of the spectrum, mainly produced by changes of the water content of leaves which can also be detected at the early stages of the disease. So, it is the physiological condition of the plant when an early infection takes place that is having a different spectrum signature and this different signature spectrum can be detected through this remote sensing devices and it can very well diagnose whether the plant is being infected by a particular pathogen or not. The Remote Sensing community defines plant disease monitoring as: detection that is (deviation from the healthy), identification that is (diagnosis of specific symptoms among others and differentiation of previous diseases), and quantification that is measurement of disease severity that is percent leaf area affected and so on. Different sensors and techniques are required for detecting plant response to various diseases and disease severity.

Here is an example of Field reflectance spectra of healthy tomato plants an infected with late blight disease. Here you can see the spectrum, top one is the healthy spectrums where as spectrums

below are depending on the disease severity and the last spectrum is mostly from the severely infected plant tissues. So with this type of spectral analysis one can establish that how much disease severity is being there because healthy being at the top and severity level is at this level so the gap can be calculated and accordingly severity of the disease can be established. So, this is another example of spectral reflectance of healthy wheat as well as wheat infected by rust pathogen. Here, also in terms of disease severity this is the healthy one this is germinated from severely infected plants. So, this spectral analysis can give us the severity level how severe is the disease.

This is a real image from wheat field where wheat crops are suffering from yellow rust in the field and with time one can see that the progression of spectrum is getting changed where the red ones are the very severely infected areas of the wheat lot whereas, moderate yellow signifies the moderately affected areas whereas, orange is the serious infected areas and green remains the healthy areas of the plant. So, from a long distance one can see that whether the field is getting affected and if it is getting affected by a particular disease how severe the disease problem is looking into the spectral pattern.

Similarly, there is another trail that is conducted with Sheath Blight of rice where 67 rice cultivators were used in a research plot and the data was collected for the remote sensing device to see what is the progression and how many varieties are getting affected by Sheath Blight (ShB) and what is the severity level of the disease in those varieties in a single experiment.

So this is the typical output image of the Sheath Blight detection by remote sensing devices where it was shown that, this is the normal RGB light play and this the HLS (high resolution spectrums) for the same plots and this is the first observation and this is the second observation. So, in the second observation certain cultivars' were severely affected by the sheath blight pathogen which can be very clearly distinguished from the spectral data that originates from the remote sensing devices. So with this even one can look

at the differences in the varieties that is responding to a particular disease like sheath blight in this particular case.

So the remote sensing device can be helpful to collect data and to get the large scale information from field such as blight in case of rice cultivars with like sheath blight pathogens.

So with this we have seen the remote sensing devices, how it works and how it can be used for collecting data at large scale and getting appropriate information very accurately and correctly at a very short time interval. So this is also a technology that can be used for other agricultural purposes for measurement of abiotic stresses or pest infestations but it is widely being used in diagnosis and detection of plant pathogens causing plant diseases.

So with this we have to an end of today's talk and in the next talk will be talking about interaction of human pathogens on plants and how they can be identified from plants and how appropriate measures can be taken after identification of human pathogens on plants. Till then have a good time.

Thank You very much.

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PDF: Remote-sensing of Plant Diseases

3 Detection of Human Pathogens on Plants

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Transcript

Hello!! Welcome to the third talk of the week that is Detection of Human Pathogens on Plants. We have seen that recently lot of human diseases are causing because of the contaminated raw foods that people consume and certain human pathogens that are being associated with this leafy root vegetables particularly, and because of consumption of such human pathogen associated plant product is casing certain ailments in human and that is why diagnosis of such human pathogens on plants is highly essential. What is the significance human pathogen? We always know that unlike most plant pathogens human pathogens that associate with plants often fail to multiply in plant host and usually occur in low numbers. But, nevertheless, they are able to cause disease in human beings despite their low population in the plant host. National and International disease outbreaks associated in human pathogens on plant products, such as lettuce, peanuts, green onions, seeds. sprouts, peppers, spices, tomatoes and cantaloupes, have occurred frequently. Current standardized assays for the detection of major pathogens on plants rely largely on microbiological, biochemical, and immunological analyses that are laborious and time consuming.

So, newer molecular- based methods, such as PCR, loop mediated

isothermal amplification or (LAMP), and metagenomics approaches offer enhanced speed and sensitivity, and some of these have already incorporated into the standard assays. So these are the tools that are used for the detection of human pathogens in plants. Certain human pathogens, especially enteric microbes such as pathogenic Escherichia coli and Salmonella species., also contaminate, colonize and even invade plants. Human diseases caused by such plant contaminants are becoming more common, widespread, and consequential, and national food safety agencies across the globe are seeking greater understanding of the mechanisms and interactions of human pathogens on plants (HPOPs). Increasing opportunities for interactions, collaborations and cooperation among plant pathologists and food microbiologists, a cross-disciplinary synergy has developed from which novel, robust and sustainable solutions to human pathogen on plants (HPOP) challenges have emerged. Fresh produce has been associated repeatedly, and with increasing frequency, with outbreaks of food borne illnesses. Particularly, green leafy vegetable, melons, sprouts, berries, tomatoes and green onions, often eaten with little or no processing steps to eliminate pathogens, are among the most common produce implicated. Shiga toxin-producing Escherichia coli O157: H7 has been found on leafy greens, then Salmonella spp. on tomatoes, peppers and cantaloupes, hepatitis A virus on green onions, Shiga toxin-producing E. coli strain O104 on fenugreek seeds, sprouts and Listeria monocytogenes on cantaloupe. These are some of the reported human pathogens strains that are found associated with such green leafy vegetables.

In many cases contamination occurs either in the field or in the processing phase. Many human pathogen on plants (HPOPs) exist in environments where plants are grown and that can be the danger of the particular phenomenon of human pathogens can be analyse through that in a single year that is (2011–2012) the Food and Drought Administration (FDA) in USA issued recalls of 56 produce items including fresh-cut fruit and vegetables and bagged

vegetables that contained Listeria spp., pathogenic E. coli or Salmonella. So this is not a small problem so this is becoming a huge problem as many of the human pathogens they are present in the environments where plants are grown or may be at the time of processing these contamination takes place.

So, opportunities for human pathogen on plants (HPOP) contamination of fresh produce begin on the farm and continue through all nodes of the food production and distribution chain, not ending till the food is consumed. So that is why so it originates in the field and it comes to the plate of the human who is consuming the food. So the chain continues till the last moment till the patient or the human is suffered from such pathogenic strains of microbes. How pathogens move, directly or indirectly, from vertebrate sources into plant foods can be a complex and multi-faceted phenomenon. Therefore, understanding these sources and pathways is critical for the development of prevention and mitigation strategies. Interestingly, the Gram negative bacterial family Enterobacteriaceae, which includes many of the human pathogens associated with plant foods (e.g., Escherichia, Salmonella, Shigella), also contains several plant pathogens (Enterobacter, Erwinia, Pantoea, Pectobacterium, etc.). So, it is very interesting that how this bacteria compete with each other because these human pathogens has to come in a competition with the plant pathogens.

The taxonomic relatedness of these plant and human pathogens raises interesting questions about the possibilities of niche competition or synergism, horizontal gene exchange in protected plant niches, or even host range expansion. So, co-existence of the human pathogen along with plant pathogen has the additional trick of horizontal gene transfer. Suppse, for example toxin producing gene from Escherichia coli, Salmonella if it is horizontally transferred to a plant pathogen like Erwinia and Pantoea. Then the plant pathogen can also release certain toxins to the plants it is infecting and thereby causing more damage to the consumer.

A few cross-kingdom pathogens such as Pseudomonas

aeruginosa, Burkholderia cepacia, Dickeya spp., Enterococcus faecalis and Serratia marcescens actually cause disease on both plants and humans. So this is another concern that some of the microbes that causes human diseases are also able to cause plant diseases. So, this is again a grave concern for all of us.

Challenges

What are the challenges because identification of human pathogens on plants is highly essential but their identification is not a easy task, so there are certain challenges. So one of those if High Background Microflora, that is – Low Numbers of Target Human Pathogens. So in a plant community as we have mentioned earlier the population of the human pathogen is normally low because the environment is not exactly suitable for their multiplication unlike the plant associated microbes. So, in a high background of different Microflora identification or detection of human pathogen has remained to be a challenge.

Perishable Nature of Plant is another problem. Some of the plant samples they get perished very quickly. So detecting human pathogens in those plants samples is therefore very very difficult.

Nonhomogeneous Distribution of Target Pathogens in Plant Samples – So that is another issue where the entire plant sample lot may not carry the human pathogen but may be a part of plant sample is carrying the human pathogen but still it causes enough danger to the consumers. Moving Target – Then it's always on move and that is why it's always a difficult task. Then, Detection and Predicting Infectivity is not also very easy. So, these are certain challenges for which it is difficult to identify and detect human pathogens on plants.

Detection of Human Pathogens on Plants – detection of human pathogens and plants are basically done through – Standard Assays like Culture-Based methods. The microbes that can be cultures easily, can be easily identified. Then Molecular methods are also used for detection of human pathogens on plants such as PCR-Based methods, DNA Microarrays and Next[1]Generation

Sequencing (NGS). Further, Biosensor-Based Techniques are also being deployed for detection of human pathogens on plants.

Microbes withdrawn from agricultural use – So, there are certain interesting phenomenon that was already prevailed and this has to be now modified or changed for example :- Pseudomonas aeruginosa is a case which is basically a Gram-negative environmental species and it is also an opportunistic microorganism. So, it establishes itself in vulnerable patients, such as those with cystic fibrosis or hospitalized in intensive care units (ICU). It has been a major cause for nosocomial infections worldwide and a serious threat to Public Health. But the same Pseudomonas aeruginosa is a very good plant good promoter and it has been used widely some years ago in agricultural crop production. So, there was every possibility that this particular microbe Pseudomonas aeruginosa will enter into the food chain and can cause disease to those people who are suffering from cystic fibrosis or if someone is admitted in the ICU. So realizing the threat of this kind of microorganism regulatory measures has been adopted and these microbes are now not recommended for use in agricultural crop production. But this is just a few examples but we have to screen other microbes also for being a potential human pathogen and only after that we can stop using those or we can deploy more sensitive methods for detection of such pathogens on the food that we consume and that is why it is important to take appropriate measures so that we can have supply of healthy and nutritious food to the consumers. So with this we have to an end of today's talk that is Detection of Human Pathogens of Plants and we have seen the significance, what is the necessity of this kind of technology, why it is important to diagnose human pathogens on plants as we have seen that certain strains of pathogen is causing severe illness to the consumers.

So with this we come to an end and in the next talk we will be talking about Plant and microbial forensics and till then have a good time.

Thank you very much.

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PDF: Detection of Human Pathogens on Plants

4 Plant and Microbial Forensics

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Transcript

Hello!! welcome back to the fourth talk of the week that is Plant and microbial forensics. We already know that forensic science is applied to investigate a crime and the same principles applied for microbial forensics or forensics in plant pathology where a criminal approach is been investigated and the perpetrator of the crime is punished. So let us see how the forensic science in Plant Pathology works.

So, Microbial Forensics is defined as the application of scientific approaches to solve a crime that involves a microorganism. Its goal is to investigate and present unbiased scientific evidence useful for attributing the crime to a perpetrator recent programs intended to enhance general capabilities in microbial forensics there have included specific attention to plant pathogens. Compared to strategies employed by traditional plant disease diagnosticians forensic applications of plant pathogen diagnostics require usually high level of stringency, reliability and prior validation. These assays must be paired with forensic investigations for example court-defensible sampling methods, chain of custody and other traditional and non[1]traditional methods of forensic investigation.

Forensics in plant pathologists – have been deployed very

recently. So, it's a blend of disciplines of plant pathology and forensic science that supports the investigation of plant diseases and pathogens by providing unbiased scientific methodology and evidence for criminal attribution. Important to this effort are trace backs strategies for determining pathogen origin and movement pathways as well as the possible role of human intent. So this is very important whether there is any involvement of human in the microbial associated crime that needs to be investigated. Multidisciplinary teams including representatives of diagnostic regulatory and law enforcement communities must work in coordination to achieve the most effective response. More creative strategies for both vertical and horizontal communication among the involved biosecurity and law enforcement agencies are needed to solve certain issues.

Now, what is the difference between Forensics and Traditional Plant Disease Diagnosis – In traditional plant disease diagnosticians are multifaceted approaches to detect and identify diseases and the causal pathogens. Here the primary stakeholders include Farmers, Extension educators, crop consultants and regulatory officials and the primary goal is to identify the pathogen to recommend effective means of disease management. Whereas, in case of forensics it is the application of scientific methods and strategies to solve a crime with the primary goal of connecting the crime to a perpetrator for the purpose of criminal attribution. The major stakeholders for forensic science include members of the law enforcement, security, investigative and regulatory communities. So these are some of the basic differences between a traditional and a forensic plant disease diagnosis.

Why do we Need Plant Pathogen Forensics?

Then what is the necessity of forensics in plant pathology. The plant based resources for any nation that includes a forests, rangelands, crops raised for food and fiber, etc. are among the most critical components of its infrastructure and it contributes to a healthy environment and robust national and international market. So, any critical element of national infrastructure might become a

target of those having a motive to harm a nation, region, company, person or other entity. And, that is why to protect this national resources one has to link plant disease diagnostics with forensics. Crimes are also always committed intentionally. Negative consequences can also result from unintentional actions. For example, if a grower purchases seed that is certified as disease free but later after losing the crop to disease learns that the order was filled inadvertently with pathogen contaminated scene there may be a cause for a lawsuit based on criminal negligence. So, it is not the grower who is responsible for such situations, but it is the packager who is basically can be charged for negligence. Other crimes may include multiple elements for example, smuggling of exotic plant material such as seeds, fruits, propagating plant parts is a frequent biocrime at airports and ports of entry, but if the smuggle material is contaminated with pathogens or other exotic microbes there may be an additional criminal charge for which forensic investigation is needed. So, biocrime is always a common problem in the port of entry, but if the biomaterial is associated with a quarantine pathogen or a new pathogen then the crime becomes more severe and it requires involvement of forensic specialists.

How we come to know that whether a crime has occurred or not?

The prerequisite to any forensic investigation is an informed judgment that a crime has been committed. It is important to know if the presence of a plant pathogen or the occurrence of plant disease may have resulted from criminal activity. So plant disease is a natural phenomenon but whether the occurrence of plant disease has any crime involved for example, it is an intentional by involvement of certain human beings disease development comes into the scenario of a crime. Since agricultural producers and consultants environmental specialists and plant disease diagnosticians are generally unused to considering the possibility of intentional intervention, a term normally called as 'suspicion inertia', it is important to consider what features of a plant disease even might prompt a contact that would lead to investigation. So it is very essential to determine whether there is any association of any crime

and then if any investigation is required to be associated with the occurrence of that particular plant disease.

Sampling for Plant Pathogen Forensic

So, sampling for forensic science involves mostly two steps – collection of sample and chain of custody.

Assay Features

And Assay features include Pathogen Detection Assays as well as Pathogen Discrimination Assays. In the pathogen detection assays the detection methods used in microbial forensics include an array of serological and molecular detection assays, mass spectrometry, nucleic acid sequencing, and bioinformatics. Selection of the most appropriate methods depends on the type of pathogen, the tools available, and the scope of the screening. Whereas, pathogen discrimination assays involved since microbial forensics is often a question of fine-level "matching" of microbes found at a crime scene with those associated with a suspect, microbes may be subjected to molecular fingerprinting techniques such as restriction fragment level polymorphism (RFLP), multi-locus variable repeat analysis (MLVA), single nucleotide polymorphism (SNP) assays, single sequence repeat or inter-single sequence repeat (SSR and ISSR) assays that will discriminate among strains of a pathogen. So, it is not only detection of the pathogen but also it is important to establish the strain or biomire of the pathogen for which certain specific molecular tools have been deployed in forensic science because this is highly essential going for a criminal investigation.

Evidence Interpretation and Criminal Attribution – is another important aspect in forensic investigations. So forensic evidence is judged in the courtroom and the judgment is conferred by a jury panel. I n the investigation of a crime that may involve plant pathogens forensic plant pathologist must gather, safeguard, analyze and interpret a comprehensive package of information to be used by prosecutors or defendants as evidence in a court of law. In addition to diagnostic assays providing pathogen identification data with acceptable level confidence levels interpretation of field and laboratory tests must be done in consideration of other evidence

such as the chain of sample custody, the history of disease site and crop, possible motives and access of individual other than the suspect and other relevant factors.

So as investigated in other human associated crime in forensic plant pathology also certain angles has to be looked upon during the court presentations. At times even a comprehensive package of evidence and court testimony is unlikely to result in hundred percent confidence in a world per day. So until and unless a full proof support system is there it is not necessarily that the perpetrator will be vindicated in the law of court if the evidences are not sufficient enough to prove the same. So with this we have seen that how important forensic plant pathology in current days and how it is helping us to stop or check certain human mediation in involvement of certain microbial associated crime and it is definitely a emerging branch on plant pathology.

So with this we have come to an end of this topic that plant and microbial forensics and in the last talk of the week we'll be talking about Development and Implementation of Rapid Detection Techniques for plant disease diagnosis. Till then have a good time.

Thank you very much.

Download

PDF: Plant and Microbial Forensics

5 Development and Implementation of Rapid and Specific Detection Techniques

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Transcript

Hello!! welcome to the last talk of the week that is, Detection and Implementation of Rapid diagnostic techniques.

We know that lot of plant pathogens that they come to a new area through transmission via seeds, and leafy vegetables are one of the major source of such transmission and several pathogens has been reported to occur in new areas and new countries through this seed export and import. So, commercial significance of seed industry also heavily dependent on this seed lot whether, it is contaminated with a pathogen or not and that is why rapid detection of such seed lots has become very very important.

Commercial significance of seeds and seed borne pathogens

Fast and sensitive diagnostic tools are necessary to screen seeds and transplants used for commercial purpose. Due to the role of seed transmission, detection methods are also important for the production of pathogen-free seeds and for their certification. It was during the past one and half decade it was observed that several new diseases were introduced in European countries in the production sector through infected seeds. Molecular methods

proved helpful in the detection of several formae speciales of Fusarium oxysporum, Verticillium dahliae and several other causal agents of foliar diseases of leafy vegetables. So, already there is a base that is why it needs to be investigated and this investigation needs to be done very quickly so that the commercial value of the seed lot is retain and the industries are not suffered and at the same time a new pathogen is not introduced to a new land. Fast and sensitive diagnostic tools are necessary so that primary inoculum vectors, such as seeds and transplants, can be screened promptly. Pathogen's detection on seeds can be a difficult task because in most cases infected seeds can be asymptomatic, making visual detection difficult or impossible. Moreover, infected seeds may be present in a limited percent, and nonuniformly distributed within a lot. Different detection assays exist for different seed-borne pathogens but only a few respect the minimum requirements for adequate seed tests. Traditionally, seed assays have been developed based on – visual examination, selective media, serological techniques, Polymerase Chain Reaction. Several new diseases of leafy vegetables for example, in case of (lettuce, wild and cultivated rocket, lamb's lettuce, cichory, endive, basil, spinach) were introduced in Europe through infected seeds. Some of them were reported for the first time in Europe or worldwide. Identifying the source of inoculum is of critical importance for effective disease management. Due to the role of seed transmission, detection methods are also important for the production of pathogen-free seeds and their certification. So, it is highly essential that such seed lots should be screened very quickly and promptly to establish whether it is carrying any pathogen or it is pathogen free. Among diseases, Fusarium wilts on lettuce, then wild and cultivated rocket , lamb's lettuce, cichory and endive (Cichorium endivia) were newly observed. This was not early observed in certain European countries prior to this. Also several species of Alternaria, of which there is evidence of being seed transmitted, are reported on leafy vegetables. For example, Alternaria cichorii is reported on lettuce, endive and scarola, while Alternaria japonica has been recently

detected on both wild and cultivated rocket. So, these are certain examples along with along with other pathogens such as, Verticillium dahliae, it was reported on lettuce, cichory, spinach and Plectosphaerella cucumerina on wild rocket, and so etc. So, lot of evidence have been gathered recently to establish that lot of new diseases has been introduced to certain parts of European countries and mostly they have been entered through the seed lots of leafy vegetables or through the transplant.

Fusarium oxysporum: Let us take the example of Fusarium oxysporum

The search for molecular techniques has been particularly intensive and effective in the case of several formae speciales of Fusarium oxysporum. So, Fusarium oxysporum has several formae speciales so it was very hard to identify which formae Fusarium is newly introduced. The detection threshold of F. oxysporum in seeds and propagation material could be increased by using molecular techniques, such as the PCR. So, PCR comes to play in such cases where the pathogen level is very low. So, PCR can multiply the pathogenic DNA to significant level for its detection.

In the case of Fusarium wilt of basil and lettuce a nested-PCR-based method allowed fast and unequivocal identification of F. oxysporum f. sp. basilici. The method permitted to detect 32 conidia/100 seeds and required 4 hours. DNA was extracted only from propagules present on the external surface of the seeds. So, technology variants of PCR's like nested PCR helped in identification of the Fusarium oxysporum f. sp. Basilica and the amount of inoculums load was very low that is 32 conidia per 100 seeds but still this technology was helpful in identifying such low amount of inoculums of the formae speciales .

Verticillium dahliae

Next, example is Verticillium dahliae – A quantitative real time polymerase chain reaction (qPCR) assay was optimized and used for the detection and quantification of Verticillium dahliae in spinach seeds, resulting quite reliable and sensitive, permitting to assess values up to 1.3 % of seed infection. So, Verticillium dahlia was also

able to detect through application of quantitative PCR and it was able to detect the presence of the pathogen as low as 1.3% of the total seed lot.

Similarly, V. longisporum has been described as a hybrid species that presents several genomic regions in common with V. dahliae. V. longisporum is a crucifer pathogen but never described in diseases associated with spinach, but in any case the external presence of this pathogen can result into a false positive; then it is important to exclude the presence of this pathogen from the samples.

So, certain other conditions has to be kept in mind, for example, Verticillium dahlia is a pathogen of spinach, but the spinach seeds can be contaminated with Verticillium longisporum which has certain genomic reasons common with Verticillium dahlia. So, PCR amplifications of those common reasons could be misinformation about Verticillium dahlia. So, that needs to be taken care of how to exclude those microbes which are sharing some common genomic reasons with the pathogenic ones but actually, not a pathogen of the concerned species.

Alternaria – Similarly, in case of Alternaria Distinguishing Alternaria species is always a challenge in leafy vegetables and PCR-based methods were used to detect Alternaia radicina infections on carrot. However, RAPD analysis that was helpful to distinguish Alternaria. radicina from the other Alternaria species. Additionally, PCR-RLFP was helpful to identify and distinguish three Alternaria species: Alternaria radicina, Alternaria dauci and Alternaria alternata from carrot seeds. Standard PCR and Real time PCR were useful in identification of seed contamination by Alternaria brassicae on cabbage and radish. So, different tools even the principal is same the variants of the particular tool is deployed for distinguishing between species of similar some pathogens like Alternaria to detect on the seed lot. So, we have seen that different tools and techniques has been deployed for rapid identification of pathogens for quick determination of whether their presence is there or not in the seed lot because it has the seed lots always carry a commercial value. At the same time seed lots need to be

checked if t is moving to a different area or a country or to another country whether, it is carrying a pathogen that it non existing to the country concerned were it is going to be shown. So, this tool s and techniques again it is coming in a big way is helping for a rapid detection of seed lots and it is helping a way in checking some quarantine pathogens as well.

With this we have to an end of the fifth week and in the last week that is in the sixth week we will be talking about how diagnostics are helping in decision making process for plant disease management. So, with this thank you very much will see you in the next week.

Thank You.

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PDF: Development and Implementation of Rapid and Specific Detection Techniques

PART VI

WEEK 6: DIAGNOSTICS IN PLANT DISEASE MANAGEMENT

1 DNA Barcoding of Pathogens of Quarantine

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Transcript

Hello! Welcome to the last week of this course of 'Detection Diagnosis and Detection of Plant Diseases' and in this course will be talking about of 'Diagnostics in Plant Disease Management'. So, the first talk of this week will be is DNA barcoding and it is very important nowadays to have a molecular signature to have proper identification of plant pathogens. Molecular signature is important because there are many closely related plant pathogens and to differentiate them from each other it is very hard to distinguish them with certain conventional technologies. So, that is why DNA barcoding has been developed to separate each other from the closely associated plant pathogens.

DNA barcoding- the necessity

So, DNA barcoding is the necessity of today and development of accurate identification tools for plant pathogens and pests is vital to support Plant Health Policies to manage plant diseases in a broader way. But there are certain Challenges: It includes increase number of plants being traded worldwide, then chance of harmful organisms spreading is also enhanced and taxonomic knowledge

on harmful quarantine organisms is not adequately available. So, these are few challenges and that is why there is a necessity of DNA barcoding for proper identification of the pathogens. The economic damage is significant: Both when a harmful quarantine organism is not identified and as well as when an organism is incorrectly diagnosed as a quarantine organism. In the European Union (EU) alone there are some 275 quarantine organisms for which protective measures against introduction into and their spread within the Community needs to be taken care of.

Barcoding for diagnostics

DNA barcoding is basically a taxonomic method that uses a short genetic marker (DNA sequence signature) in an organism's DNA to identify it as belonging to a particular species. So, this DNA sequence signature is basically the identity of that particular pathogen. Although the DNA sequences of related species are generally very similar, there are differences to be found. The part of the DNA sequence that is different is specific to that particular organism and forms a unique and specific molecular DNA barcode. One or several specific DNA barcodes are made for each quarantine organism and that is how we are now able to regulate the quarantine pathogens.

Plant Pathogen Barcode (PPB)

It is to facilitate the rapid and accurate identification of plant pathogens, a new database is developed that firmly link names and associated meta-data to a rapidly developing new standard in biology. DNA barcoding is an emerging gold-standard for species recognition. This development has already shown itself to have unprecedented power for clarifying species identities and limits, uncovering new and often cryptic species. So, it has been very useful to even identify new or even cryptic species. In response to the growing number of researchers who are using barcoding, data standards for barcode records have been developed, and an open access database has been created. The Consortium of barcode of Life, in short it is known as (CBOL) has engaged more than 125 Member Organizations from 40 countries.

QBOL (www.qbol.org)

So, QBOL is an European Union (EU) project on DNA barcoding, that started in 2009 to generate DNA barcoding data of quarantine organisms and their taxonomically relatives to support plant health diagnostics. The data are included in a database, called Q-bank (www.Q-bank.eu), which now consists of a dynamic open-access database of quarantine plant pests and look-alikes, linked to curated and publicly accessible reference collections. It contains sequence and morphological data including photographs, nomenclatural and diagnostic data of specimens available in reference collections for the benefit of the users. QBOL made DNA barcoding available to plant health diagnostics and focused on strengthening the link between traditional and molecular taxonomy as a sustainable diagnostic resource. Within QBOL, collections harboring plant pathogenic Q-organisms were made available. Informative genes from selected species on the EU Directive and European Plant Protection Organisation (EPPO) lists have been DNA barcoded from vouchered specimens and the sequences, together with taxonomic features and have been included in a new internet-based database system i.e. Q-bank: www.q[1]bank.eu.

DNA barcoding of fungi – So, DNA barcoding of fungi, incase of fungi it was mostly the ITS amplification and sequencing was done, but it was helpful to segregate some of the plant pathogenic fungi nut in certain cases ITS alone was not sufficient and for that other signature genes has been sequenced and DNA barcode has been developed accordingly. For, example incase of Didymella ligulicola it is the ACT that is required for DNA barcoding whereas in certain cases it is the TEF, TUB, or it the COI. These serve as DNA barcode for segregating these pathogens in a unique way from other related species.

DNA barcoding for bacteria

Similarly, DNA barcoding for bacteria, DNA extraction was done and it was followed by 16S rRNA sequencing and it gave us segregation of species like Clavibactor, Rolstonia, Xanthomonas, Xylella. But for other specific identification one has to go for GyrB,

Muts, Gyrb and Muts and that gave us different signatures for certain other bacterial species and they were differentiated from the closely related ones.

DNA barcoding for nematode

DNA barcoding for nematodes also, it was the ribosomal DNA that was sequenced and it was able to differentiate nematodes from each other from different geographical areas.

DNA Barcode Library/Database/Informatics

So, all these informations had been deposited in the Q-bank and it has served as a library and database for other informatic applications. So, DNA barcode library, database and informatics is again a very useful source for identification of quarantine pathogens and it has been now made available to all the uses so that the global communities benefitted out of this database. The database was developed within the Dutch FES project during 2006–2010 and has been further developed during the QBOL project. Six databases were created: i.e. for fungi, arthropods, bacteria, nematodes, viruses and phytoplasmas. The total database Q-bank is freely accessible via internet (www.q-bank.eu) and a software module to export to and import from Genbank (and therefore EMBL) has also been implemented. The Internet-based software is continuously improved to comply with the needs of the end-users. So, filling of the databases has been made significantly during last stages of the QBOL project. So, it has been initiated and then it has been improved and now it is a very strong and powerful database for identification of quarantine pathogens.

So, once the database is publishes it helped internet visitors to regularly use the system. Websites are therefore not restricted to the users participating in the QBOL project anymore. Usage of the different databases are monitored by Google Analytics and that is how one can presence the importance and significance of the database by looking into the number of users. The bioinformatics and databases of Q-bank are based on the BioloMICS software (BioAware, Belgium). This tool allows specialized and scientific biological databases to be created to fit the specific needs of

researchers working on any organisms for example arthropods, bacteria, fungi, nematodes, phytoplasma and plants viruses. It is used by a broad base users such as taxonomists, ecologists, human, plant pathologists, molecular biologists, pharmacists, industrial researchers, and so on. This again shows the significance and importance of database. The database is thus helping to identify quarantine pathogens and taking adequate measures to contain them.

So, in this particular talk we have seen that how DNA barcoding is helping pathogens to be detected, For that, we have to go for specific molecular signature and it has been assigned to a specific pathogenic micro organism and the information needs to be deposited in a public domain so that it can be accessed by all potential users and they can contain and again identify the quarantine pathogens and various levels and contain them and take adequate necessary measures. So, with this we come to an end of this first talk and in the next talk we will go for on-sites testing and moving decision making from lab to the field. So, till then have a good time.

Thank you very much.

Download

PDF: DNA Barcoding of Pathogens of Quarantine

2 On-Site Testing: Moving Decision Making from the Lab to the Field

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Transcript

Hello! Welcome to the second talk of this week that is 'On Site Testing Moving Decision Making from Lab to the Field'. So, this is a very important talk in a context that detection and identification usually very critical for containment of a particular pathogen and to protect the growers from severe losses. But usually the process takes longer time when it comes from the field to the lab conditions and then decision making is done on the lab basis. If the decision making system is changed from the context that the decision making could be obtained in the field condition then certainly it is going to save time and help the decision making process.

So 'On-site testing' basically is a term that is often used to describe two distinct activities, Firstly detection that is the initial locating of the pest or pathogen infected sample which is in most instances is performed visually. The second is activity is identification, and it is usually achieved by sending suspected samples to a laboratory. So, providing technological solutions to enable more rapid decision making is a must nowadays. It is not necessarily just inspection services who benefit from these

techniques, they can be deployed throughout the farm to fork to limit losses caused by pathogens. How best to deploy detection methods is however a matter for policy makers and other stakeholders. Deploying simplified detection and identification methods remotely helps to speed up inspection as well as facilitates trade. So, that is why on-site testing is gaining popularity and devices have been developed to make decision making process in the field itself.

Performing diagnostics is part of a decision making process to prevent or limit spread of pathogens. The faster the decisions are made more effective the action may be. Traditionally once a potential disease has been located, samples are sent to a laboratory for testing that causes delay in the decision making process. Furthermore, If pathogens go unnoticed at pre[1]symptomatic infection stages, this failure of visual observation can lead to its spreading unchecked until it has built up to such a level that it can be seen. So, decision making at field level is very significant. These issues have led the drive to develop technological solutions that would fulfil two complementary roles.

Firstly, putting tools into the hands of those on the front line to enable rapid identification of pathogens and it would prevent delays.

Secondly, developing detection tools that guide those on the front line to the site of the problem, at the pre-symptomatic infection. So these tools are very vital for development for technological solutions at the on-site testing. These tools when linked together enable a more efficient detection and diagnosis process enabling faster deployment of control measures. Definitely, faster deployment of control measures would lead to least losses by the growers. Methods based on latex agglutination have been performed for plant diseases since in the early 1980s. For example Ani Biotech has developed a potato virus test kits by Ani Biotech on the basis of latex agglutination method. Since then more refined methods have been developed to enable rapid identification. Early test kits based on latex agglutination on glass or plastic slides,

required:a large number of temperature labile reagents, had multiple steps in which reagents were added sequentially, and the interpretation of the result was often subjective, requiring a fair amount of training and experience to the reproduce.

Some of these second generation kits (e.g. Alert kits by Neogen) also incorporated chemical substrates, effectively recreating laboratory ELISA methodology, yet performed rapidly on a solid support. This provided advantages in terms of both usability and interpretation of results which were no longer subjective and easily interpreted by non-specialists even in the field situation. So, this is a technological advancement that is how it is helping on the on-site testing or decision making at the field level.

The most significant innovation came in the late 1990s with the application of homogeneous test kit formats developed and exploited. The Lateral Flow Device (LFD) format was exploited initially in the phytodiagnostics arena for the detection of potato viruses for use in seed certification systems and proved to be a considerable improvement over previous formats. The underlying chemistry in an LFD is effectively the same as a latex agglutination kit, the accumulation of antibody coated latex or may be colloidal gold particles caused by the presence of the antibody target. The key difference however is that the binding occurs during the capillary flow of sample and reagents along a membrane, but not in the solution as it was in the earlier cases.

Testing based on LFD technology remains the simplest and most rapid option for field use where specific binding reagents for the targets of interest are available. The only significant drawbacks to LFD approaches to field detection are the availability of reagents with a specificity appropriate for the application and the inherent lack of amplification that limits sensitivity. For simple pathogens such as viruses and to some degree bacteria and fungi, antisera or monoclonal antibodies with a useful level of specificity are often available, but for more complex targets this is often not the case. So, LFD approaches has certain limitations as we have seen that. It is good for of certain simple pathogens like fungi, bacteria and

viruses but if it is a complex disease then definitely these LFD's are not sufficient help the diagnosticians under field condition.

In-Field Identification

So, In-Field Identification where applications of LFD's technologies are used very quickly they can collect the plant tissues and they can get the tissue macerated and the tissue extract can be then subjected to the LFD device and within few minutes the presence or absence of the specific bands can help us to identify that whether the plant is affected by the organism being tested is there or not. Similarly, there are other devices which require no electricity rather a movable or portable water bath or a heat block can be used to go for amplification test and then subject to its analysis and this again help us to take decision in the field itself but without coming to the laboratory. For greater sensitivity and specificity, molecular biology methods such as PCR is used to amplify target nucleic acids. However, implementation of these methods on-site has been a challenge. A number of companies have produced fieldable real-time PCR equipment. Whilst portable real-time PCR has been evaluated extensively there are a couple of significant drawbacks to it implementation. Firstly, in PCR methods, extraction of nucleic acid generally requires reasonably elaborate extraction methods to avoid co-purification of compounds which inhibit the enzymes. Secondly, whilst rugged, portable and in some cases battery powered equipment is available it remains expensive, largely due to the need for careful temperature regulation and sensitive detectors that are required for its detection. To solve both of these problems subsequent research has been focused on evaluation of isothermal amplification chemistries, which could eliminate both the problems associated with the normal PCR reactions.

Isothermal amplifications are methods in which the amplification reaction is incubated at a single temperature. This gives advantages in terms of simplicity over PCR, since the reactions do not need to be cycled accurately between temperatures, thus water-baths, dry-blocks or incubators can be used to incubate reactions. Plant

pathogen assays by the Loop mediated AMPlification (LAMP) method is the most widely adopted method to date under field situation.

Finding Pathogens – to finding a pathogens require a sense of having a plant infected by a pathogen which can be spelled out as: Sniffing Pathogen Infection. Then one must see the infection from a distance and one must use Surveillance Tools for detection and diagnosis of the possible occurrence of plant pathogens. So these small equipments that are coming up that are replacing some high sensitive equipments like PCR and these are helping the investigators to take a decision at the on-site level without taking back the samples to the lab for its verification. It is only in certain cases where on-site testing is not confirmatory only then the samples are then brought back to the laboratory for further analysis. So, this is how one can see that how decision making processes has been now moved from the lab to field. Based on development of these technologies like LFDs and isothermal amplifications processes. With this we have come to an end of the topic On-site testing and decision making from lab to field. In the next we will be talking about virtual diagnostics networks and we will see how it s is helping any investigators or growers to take a decision for plant disease management.

Thank You.

Download

PDF: On-Site Testing: Moving Decision Making from the Lab to the Field

3 Virtual Diagnostics Network

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Transcript

Hello!!Welcome to the third talk of the week and i.e Virtual diagnostics and network. Plant is always at risk because of several reasons one of the most significant reason is plant pathogen. And some of them be originated in the same level, or same area or whereas, some of them has been identified to be coming from a distant places or from a newer, another country. So, it is always important to have a knowledge base on most potential threats to crop plants because if the pathogen is not existing earlier in a particular area people may not be aware of the pathogen which maybe of quarantine significance from of that area without proper database or without a proper tool to go for identification. So, in this context virtual diagnostic networks come into play a big role and it helps the growers and decision makers into a great extent.

Plant Health Risk

The containment and mitigation of exotic plant pathogens are dependent upon early detection and accurate diagnoses also in a timeframe to enable effective response. Increase dependency on international trade to address global economic and food security challenges pathogen incursion will also continue. But a question arises is that do we have a rational plant biosecurity strategy and

adequate plant biosecurity infrastructure to protect plant systems from the pathogens that threaten the plant health? The emergence of hybrid Phytophthora species has been reported as a consequence of the global trade in nursery and landscape plants. Of great concern is that the host range and aggressiveness of the hybrid Phytophthora species were not predicted based on the parental phenotypes. OS, where situation like this occurs then it is definitely useful to have a broader database or broader level of information to actually detect and identify and diagnose the appropriate problem that is associated with the plant disease. Similarly, novel virulence patterns in plant pathogenic bacteria have been attributed to horizontal gene transfer (HGT). The global movement of plants may provide more opportunity for HGT among plant pathogenic bacteria creating challenges for plant health in general, and plant diagnostics, in particular. So, when a bacteria acquire a new gene from a closely associated or bacteria, then bacteria turns out to be a different variant from the original strains that are present in a particular locality and that is a bigger challenge again to identify such pathogens with genetical variations and for this we need to have informations on this regard. The ability of cryptic satellites to alter the host range of Gemini viruses may present new challenges to plant health and confound our ability to diagnose and mitigate these new virus diseases. So, this shows that whether it is a hybrid fungal pathogen, or whether it is a horizontal gene transferred pathogenic bacteria or whether it is a satellite variated changes in viruses. These all possible occurrences of new strains of viruses , fungi and bacteria could help us to identify based on the existing database that is available that can guide somebody to help and diagnose the problem. The

Need for Robust Plant Diagnostic Systems

So there is need for The Need for Robust Plant Diagnostic Systems. With respect to trade, the mere presence of a pathogen can stop shipments whether or not the disease was manifested. Being able to accurately identify a pathogen and diagnose a disease is essential to ensure proper disease mitigation measures are

implemented and to ensure that correct trade decisions are made. So, mere detection of a pathogen in a shipman can stop the shipman from moving from the port and it may cause a severe or tremendous loss to the to the industry or to the traders but a quick decision is required to be obtained to minimise such kind of losses. With respect to sensitivity, specificity, and robustness, the stringency of diagnostic technologies and protocols vary across the continuum from pre-infection detection to post-outbreak monitoring. So it is highly essential for: Prevention, Early Detection and Accurate Diagnoses, and Response and Recovery and this can be achieved when we have a very robust plant diagnostics system.

Virtual Plant Diagnostic Networks

Virtual Plant Diagnostic Networks can play a bigger role in this aspect: A global network of plant diagnostic infrastructure is needed to facilitate the detection, diagnosis, and management of recurrent, emergent, and introduced plant pathogens. So data that is available in one part of the globe, if it is not shared with the other part of the globe then if a new pathogen emerges in a new locality, then they have to face a greater challenge to identify the pathogen. But if the data is shared then very quickly one can go for proper diagnosis and other identification of the possible plant pathogen. Trade increases the risk to plant health as a direct result of the movement of unwanted plant pathogens and pests. The International Plant Protection Convention (IPPC) requires that signatory nations abide by strict Sanitary and Phytosanitary (SPS) standards to minimize the movement of plant pathogens and pests across national boundaries. However, the systems to ensure that only pathogen-free plants are put into global distribution chains are overwhelmed by the massive volume of plants that are shipped over great distances in short period of time. So, its a big challenge and in a global trading system a huge amount of plant material being shipped in a very shorter time frame so to detect and diagnose time frame moving along with those plant samples is a very very tough job.

Plant Diagnostic Networks

Plant Diagnostic Networks has been developed. In 2002, the Land Grant University (LGU) system in the U.S. partnered with the United States Department of Agriculture to create the National Plant Diagnostic Network (NPDN) to increase the capability and capacity of plant diagnostics throughout the U.S. This mission of NPDN is to promote the early detection of plant pathogens and pests by training first detectors and ensuring accurate and rapid diagnosis. The U.S. Agency for International Development partnered with a few LGUs to create the International Plant Diagnostic Network (IPDN) to extend this concept to nations where limited diagnostic resources are available. So, it is a very good initiative in the sense that the Land Grant Universities along with the USDA in United States of America created the National Plant Diagnostic Networks and they have extended this network to a global platform in the International Plant Diagnostic Networks which can be used by those nationa's where limited diagnostics resources are available.

The Global Plant Clinic (now called PlantWise) was created by CABI to bring plant diagnostic support to rural communities in low income nations. PlantWise has offered on-site clinics in rural settings in South America, Asia, and Africa and where internet connectivity exists, PlantWise offers on-line digital diagnostic support from the United Kingdom itself. So, again it is a global platform that is known as PlantWise. It is giving service to Southern America, Asia, and African countries and where plant doctors are going to the farmers, meeting them and helping them to diagnose the problems and they can also give service right from the United Kingdom if the internet connection is available at the local level. The European Union Framework programs funded multinational efforts to create a virtual biosecurity research and diagnostic network for Europe. The concept is to link researchers and diagnosticians across Europe to a mutually beneficial network to enhance plant diagnostic capabilities and providing first detectors in the field with access to information necessary to ensure early detection of emerging or introduced plant pathogens. So, Plant Health Australia is working to establish a national plant diagnostic network with

many of the same goals as the other networks. Scientists in Australia have created an on-line plant biosecurity toolbox (http://old.padil.gov.au/pbt/) to provide access to diagnostic resources and have already deployed a digital diagnostics system (http://www.padil.gov.au/Rmd) for Australia that now extends that capability into Southern Asia. So, apart from United States or United Kingdom, it is the European Union along with Australia that all are developing global platforms and these platforms are helping and reaching to small farm holders and helping them to make decision on their farm side to take appropriate control measures for saving their crops.

Magnitude of the Challenge for Plant Diagnostics

So, Plant diagnosticians have responsibility for a vast diversity of host plant species often spanning four levels of taxonomic complexity (e.g., varieties, species, genera, families). Each one of these plant species has a vast diversity of pathogens that cause an array of diseases, each with its own set of host specificity and ever-changing systematic of the pathogens. So, these causes a heavy problem for the plant diagnosticians because they have deal with a vast varieties of plants, and vast varieties of pathogen that are specific to certain hosts and they are not having bought hosts rents. So, they need to have been better equipped in terms of knowledge and information about those plant systematic as well as microbial systematic. Plant diagnostic laboratories at air and sea ports have enormous challenges with respect to the number of shipments, the number of samples to be processed, and the very short timeframes within which diagnoses must be rendered. So, its again a big challenge that in sea and airports huge number of samples comes and the diagnosticians have to perform the diagnostic tests of this large number of sample within a very short span of time. Some plants and plant products have short shelf lives and consequently, any delays can decrease the value of the plants. So, this is another challenge to how to deal with such plants or plants products which has very low shelf life. So, therefore a concept of life Virtual International Plant Diagnostic Network has come up.

Concept for a Virtual International Plant Diagnostic Network (VIPDN)

Few national and regional plant diagnostic networks are functioning very well and connecting those national and regional networks through a virtual international plant diagnostic network (VIPDN) is proposed. The mission and function of a VIPDN was: to provide a mechanism to facilitate the exchange of non-trade sensitive diagnostic information and resources, and to provide a vehicle for cooperation among plant diagnosticians around the world, and to provide a directory of plant diagnostic laboratories to facilitate interaction with the global research community. So, the concept of virtual international plant diagnostic network is highly appreciated as it has given the opportunities to the people concerned to have information at global level. Any information that is available at any corner of the world can be accessed through this platform. Sharing of plant diagnostic information and expertise around the world would be of great value to the international plant diagnostic community. A VIPDN could be the platform for sharing diagnostic resources such as images of symptoms, primer and probe sequences, the advantages and limitations of specific diagnostic protocols, technological expertise, and so on. So, virtual diagnostic network is now becoming a reality. And now it has to be a question of time that how it is linked to all the available databases so that access of all the available resources could be made from all the corner of the world. So, with this we have seen that how virtual diagnostic network are going to help us in future to detect, identify and diagnose the pathogens or its biomars or its specific strains and then with this informations the growers can be really be helped by providing adequate resources and management practices to the crops that are suffering from a particular plant, pest or pathogen. With this we have come to an end of this talk of virtual diagnostic network. In the next talk, we will talk about 'Use of Airborne Inoculum for Detection and Disease Management' decisions. Till then…

Thank you very much.

Download

PDF: Virtual Diagnostics Network

4 Use of Airborne Inoculum Detection for Disease Management Decisions

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Transcript

Hello!! Welcome to the fourth talk of this week that is "Use of Airborne Inoculum Detection for Plant Disease Management Decisions". We all know that the vast amount of plant pathogens they produce spores and these spores are mostly airborne in nature. So, detection of airborne inoculum of a particular pathogen can help us to take a decision that whether a disease is going to occur in a shortly or not. So, let us see how it is helping us to take a decision. Knowledge of inoculum presence has been used for decades to help guide disease management decisions. However, its implementation on a broad scale has been limited due to the capital cost and requirement of technical skill for effective monitoring of plant pathogen presence across large areas. Recent advances in nucleic acid detection technologies are showing promise in enabling field level implementation of inoculum detection and quantification to aid in disease management decisions. And, this is the typical inoculum trapping device and that is put in field situations and the spores it collects then transferred for detection and diagnosis at molecular level.

There have been several success in monitoring airborne inoculum to aid in disease management systems for example presence of airborne inoculum was used to initiate fungicide application to manage potato early blight caused by Alternaria solani so, this was a successful example. Similarly, the hop downy mildew disease forecaster system is used to in the area of Hallertau regions in Germany to timely application of fungicides. This system relies on a combination of weather based disease forecasting and the visual identification and quantification of Pseudoperonospora humuli sporangia to guide timing of fungicide application; thus, it demonstrate that the monitoring airborne inoculum can be commercially implemented. So, this successful examples has enough to say that this technology needs to be deployed for inoculum detection so that timely application of fungicides can be taken place. While inoculum monitoring can be useful for aiding the disease management decision it has always been difficult to implement on a broad scale due to the difficulty in and cost of sample collection and visual identification of infective propagules. So it has some challenges and these has to be managed accordingly to reduce the time required for assessing samples and increase confidence in inoculum identification various amino logical and nucleic acid-based technologies have been developed that are suitable for detecting and quantifying airborne inoculum.

Epidemiological concepts for monitoring airborne inoculum

So, epidemiological concepts for monitoring airborne inoculum – disease management strategies of airborne plant pathogens are based on the assumption that inoculum will always be present and often failed to predict that the severity of the epidemics because they do not account for quantity of initial inoculum present at the location. So, the assumption are that there will be always in the presence of the inoculum and the inoculum presence will be normally in a higher level for causing a disease in the nearby plants. There are numerous regions for differences in initial inoculum: it may be due to the microclimate, or it may be due to the management practices that impact inoculum survival and the

amount formed by the previous season inoculum. So, different factors may be associated and these factors may play a significant role in determining the inoculum level but at times this is also a challenge for the diagnosticians to accurately assess the environmental implications and presence of inoculum load. For example, the grape powdery mildew cleistothecia are considered the predominant overwintering structure and are formed in late summer to early fall. The amount form is considered to be the function of the disease severity and influenced by canopy density and microclimate, which result in the aggregation of overwintering inoculum. So in case of grape downy mildew it is the cleistothecia that is responsible for causing the disease and it is not only the cleistothecia that is present in the canopy but it is the structure of the plant micro environment, it is the pranker canopy and all these factors come into play a role in governing the disease severity by the pathogen.

Methods for Monitoring Airborne Pathogen Inoculum

So, methods for monitoring airborne pathogen inoculum includes the practical assessment of airborne inoculum presence requires a means of collecting airborne propagules that is both easily processed and inexpensive .There are two main approaches for sampling airborne inoculum that is passive sampling and volumetric sampling. In passive sampling it relies on either gravitational forces to cause settling of airborne propagules to horizontal surfaces that is coated with glass slides or agar plates in the area of interest or inertia to impinge particles onto a vertical surface. Although quite cheap and easy to implement the highly variable sample volume associated with passive sampling strategies limits their utility in monitoring for pathogen presence. This approach also tends to utilize a large sampling surface which can be advantageous for visual detection but poses problems for other detection methods. The volumetric samples utilizes three main approaches that is inertia, filtration and cyclonic or centrifugal separation to collect propagules by moving either volumes of air over the sampling surface or by moving the sampling surface at a known rate through

the air to cause impaction of airborne propagules onto a or in sampling matrices. Thus, achieving a standard air sample volume electrostatic charge has also been used to collect airborne propagules onto a sampling matrix. So, these are the two basic approach that are used for monitoring airborne pathogen inoculum that is the passive sampling as well as the volumetric sampling.

Methods for Pathogen Identifications

The methods for pathogen identification after sampling involves immunological testing nucleic acid testing and isothermal amplification. All these techniques are capable of delivering and appropriate answer to the questions like what type of pathogens inoculum is available at a current location, at the current season, and what threat it is going to cause in the coming weeks or month on the crop that is being cultivated in and around the area. So airborne inoculum monitoring is a very specific method of detection of plant pathogens and once the spores are trapped either through passive or volumetric methods they are then subjected to immunological nucleic acid or isothermal amplification process for its accurate diagnosis and then to recommend appropriate recommendations for control of that particular disease to occur in that particular locality. So with this we have come to an end of the topic that airborne inoculum monitoring how it helped us in detection of the threat of the pathogen that may cause certain disease in that particular locality. And in the last talk of this week we will be talking about plant disease diagnostic capabilities and networks and how it is helping us to manage plant disease in a greater way. Till then have a good time.

Thank you very much.

Download

PDF: Use of Airborne Inoculum Detection for Disease Management Decisions

5 Plant Disease Diagnostic Capabilities and Networks in the Diagnosis of Plant Diseases – The Path to Effective Control Measures

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Transcript

Hello!! Welcome to the last talk of the week as well as of the course that is plant disease diagnostic capabilities and networks. We all know that plant disease detection and diagnosis is so vital to save the crop from getting incurring severe losses but at the same time we need to have capable resource persons available to perform the task and networking of resource persons is always a useful tool to provide solutions to the end users that is the small farm holders. So, plant disease diagnostic networks have developed worldwide to address the problems of efficient and effective disease diagnosis and pathogen detection, engendering cooperation of institutions and experts within countries and across national borders. Networking maximizes the impact in the face of shrinking government investments in agriculture and diminishing human resource capacity in diagnostics and applied pathology. New technologies promise to improve the speed and accuracy of disease diagnostic and plant pathogen detection. Widespread adoption and standard operating procedures and diagnostic laboratory

accreditation served to build trust and confidence among the institutions.

Why Diagnostics Matter?

It is well recognized that threats in invasive pathogens to plants, whether crops, horticultural commodities, or members of natural communities such as forests and grasslands are increasing as a result of globalization, increased human mobility, climate change and pathogen and vector evolution. Taken in total with damage caused by emerging, re-emerging and chronic or endemic pathogens, the potential for economic loss is significant in plant systems. The food security is threatened in resource-poor countries during these epidemics in stable crops and income generation from opportunities to exploit new and emerging markets is curtailed. Disease diagnosis and pathogen detection are central to our ability to protect crops and natural plant systems and are crucial prelude to undertake prevention and management measures. So, that is why diagnosis of plant pathogen is highly essential and it does matters to us.

Plant Safeguarding and Biosecurity

The responsibility of safeguarding plants against invasive pathogens is held officially by national plant protection organizations (NPPOs). In addition to their regulatory functions, NPPOs conduct pathogen surveillance and pest risk analyses, inspect, treat and certify export products, inspect and, if necessary mitigate risk on imports and share information on pathogens and regulations.

Disease Management Decision Making

So, disease management is a decision-making process and it is very essential to have a decision before we adopt for a particular disease management practice. Diagnostic networks are crucial in conducting large-scale monitoring programs, surveillance may be done by established networks or by networks organized for monitoring a specific pathogen and disbanded after completion of the program. The example of soybean rust illustrates the role of diagnostic networks in pathogen detection diagnosis and

surveillance. Phakopsora pachyrhizi, the causal agent of soybean rust was initially listed as a select agent before its recent entry into the United States. It's an economically devastating disease and their diagnostic network was established to identify the pathogen in new localities in the United States. The diagnostics tests included a real-time PCR assay, an immunofluorescence spore assay and a field-usable lateral flow immunoassay were developed and tested. Surveillance and monitoring well accomplished utilizing a network of sentinel plots and spore traps, tied into web-based reporting and communications. Thousands of farmers and agronomic professionals were trained as the first detectors. This is how the capability of the first detectors were enhanced to check and monitor the progress or occurrence of a newly emerging pathogen in the United States.

Diagnostic Capacity

Diagnostic capacity-building involves training of the human resource and it is common and easily recognized disease are often diagnosed by an astute individual, who may be trained diagnostician, experienced farmer, extension educator or consultant familiar with the crop. However, unlike human and veterinary medicine, trained practitioners in plant pathology are a relatively rare commodity and clinicians with appropriate training and access to necessary infrastructure and technology to diagnose a broad range of pathogens afflicting plants are particularly scarce. So, this is a challenge and that is why we need to increase the base of human resources to enhance their capacity to diagnose possible pathogens that cause diseases in the particular area. Human resource development in plant diagnostics in the developing world has generally lagged behind then that of the developed countries.

Technology

The technologies that are involved during capacity building – capacity for traditional pathogen identification is generally insufficient to meet needs in both developed and developing countries, therefore the certain high-tech tools for pathogen diagnostics have expanded at a rapid rate. Field-ready serological

tests such as lateral flow devices are commonly used as diagnostic tools to aid disease management decision-making, to back up diagnosis based on symptoms and as it triage tool to pre-screen plants for specified target diseases. For example, an LFD for Phytophthora species detection has proven to be significant value in the United Kingdom for pre-screening woody plants for the absence of Phytophthora ramorum or Phytophthora kernoviae at the time of inspection. Samples testing positive are then sent to laboratory for follow-up testing with more specific lab tests such as PCR and pathogen culture. So, technologies like (LFDs) are coming in a big way to help in the decision making process. So, in case of United Kingdom it was Phytophthora species that was causing sudden death in the oak plants and initially althoughLFDs has given some indications of the pathogen then based on those indications that samples were sent to the lab for final confirmation and with final confirmation then their appropriate corrective measures were adopted.

Infrastructure

Then infrastructure is also equally important just like technology and human resources. Visual examination, microscopy, culturing, a few simple biochemical tests and ELISA are the mainstays for most routine diagnosis. When coupled with diagnostic references such as disease compendia, pathogen-specific manuals, image databases, these techniques in the hands of trained diagnosticians and specialists are sufficient to provide answers in a reasonable amount of time at a manageable cost. So, simply in the involvement of simple or sophisticated equipment may not lead to an answer at times so that has to be supported with other information such as availability of disease compendia, pathogen specific manuals and image databases which greatly helped the are diagnosed stations to go for specific diagnostic tests. Many diagnostic networks build and maintain expertise database within their communication system to facilitate knowledge sharing. For example, European plant protection organizations supports a searchable database on its website containing a list of laboratories by country and an expertise

list by pathogen. Experts can be contacted via email by network members upon entry to and access code. So, diagnostic networks can again help the diagnosticians in other part of the world. If these diagnostic platforms are made available to each and every worker then they can get access to even experts and get a proper feedback on their queries.

Plant Disease Diagnostic Networks

So, plant disease diagnostic networks in the United States they have National Plant Disease Diagnostic Networks. In the Mediterranean and European Plant Protection Organization (EPPO) has expanded from 15 to 50 member countries because of its usability and usefulness. Then Global Plant Clinic (GPC) are also coming up and the global plant clinic it is a consortium of CABI Bioscience, Rothamsted Research, States resource and Central Science Laboratory, United Kingdom. The Global Plant Clinic provides a cost-free diagnostic and advisory services for NPPO's in developing countries that provide diseased plants samples. The GPC initiated the establishment of mobile plant health clinics in several developing countries. So plant health clinics fulfill an advisory role in a cost-efficient and locally operated manner. They occur in public places such as market on a regular basis where growers routinely arrive with disease plant samples. The clinics offer reliable advice on routine plant health problems affecting any crop and differentiate symptoms due to a biotic and biotic stresses. So, global plant clinic is a bigger platform, a bigger network led by CABI bioscience, United Kingdom and it is also providing assistance in the form of mobile clinics where a grower can bring their samples to the plant doctors and they can be given proper advice looking into the symptoms that is another characteristics of the disease plants.

International Plant Diagnostic Network

The International Plant Diagnostic Network (IPDN) was initiated in 2005 with a goal of fostering development of local capacity for diagnostics through establishment of communication and data sharing networks, training in classical and modern diagnostics and research into new diagnostic methods. The IPDN model after the

United States and NPDN, and as with that network is comprised of regional hub, local satellite diagnostic laboratories. Three regional programs have been established to date; in Central America, it is the hub lab in Guatemala coordinated by a private company; Agroexpertos, East Africa a hub in Kenya coordinate by that Kenya Agricultural Research Institute and in West Africa the hub in established in Benin and is coordinated by IITA.

The important objective of IPDN is training diagnosticians in basic and advanced diagnostic methodologies. Reporting new disease through International outlet is highly encouraged and the same was done in West Africa regional program where they have reported the occurrence of Ralstonia solanaceraum, the causal agent of devastating wilt disease of tomato through the Benin. So, this is how the International plant diagnostic network is working and it's coming up and people are taking help of these diagnostic networks and that is how the emphasis has been given that more number of resource persons are available at various levels through capacity building and training for them for proper diagnosis and followed by support by a international database of global plant clinic database which supports them with necessary information and images and the plant doctors can very well take help of this necessary information from this international networks and then they can help the local growers through either advisor mode or through the mobile clinics. So, with this we have come to an end of the week six and with end of the course 'Detection Diagnosis and Plant Disease Management'. I am sure you all have gained comprehensive understanding on the methodologies that are used to detect and diagnose plant pathogens and how this detection and diagnosis is helping us to take a decision on management aspects of plant pathogens and thereby, it is preventing entry of quarantine pathogens, as well as it is also preventing loss during the international trade, and it is also preventing loss to the growers at their own farm. So, I am sure you have enjoyed this course thoroughly and I wish you good luck with your future endeavors.

Thank you so much for being with me for this last six weeks.

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