

Collar rot - rotting of the stem at or about soil level

Foot rot – rot involving the lower part of the stem –root axis

Gummosis – a plant disease having secretion of gum as a well-marked symptom. Common in Eucalyptus and other woody plants

Mottle - arrangement of spots or confluent blotches of colour, often symptomatic of virus diseases

Ring spot - a circular area of chlorosis, the centre remaining green, symptomatic of virus diseases

Albinism - Plant tissues becoming white

Vein clearing - Leaf veins becoming whitish or translucent

Interveinal chlorosis - Leaf veins remain green but tissues between veins become chlorotic. Lesions – areas of diseased tissues. Localized lesions are restricted areas of diseased tissues.

Mummification – initial rotting of fruit by fungal pathogen transforms the fruit into a dry shrivelled “mummy”. Highly resistant to decay by other organisms. Serves as a means to “overwinter”.

Defoliation and fruit drop – In some plants, the leaves drop prematurely because of disease; and some plants have premature fruit drop. Sometimes, leaf drop and fruit drop indicate terminal symptom (going to die)!

Blast: The sudden death of shoot and flower. Leaf lesions begin as small whitish, grayish or bluish spots. E.g: paddy blast – *Pyricularia oryzae*

Leaf spots: Spot of dead cells or the presence of small necrotic areas on leaves. This is may be due to virus, fungal, bacteria, air pollution, insect bites etc.

E.g. Tobacco ring spot – virus, Celery leaf spot- *Septoria apiicola*; Pea nut leaf spot – *Cercospora personata*; Angular spot of cotton – *Xanthomonas malvacearum*.

Anthrachnose: Normally infected by imperfect fungi or Ascomycetes. Produces blackish lesions. Blackness being due to the presence of dark spores, mycelium, or both.

Caused mainly by species of Melanconiales in the genera of *Colletotrichum*. E.g.

Anthrachnose of *Phaseolus* sp – *Colletotrichum lindemuthianum*;

Chili anthrachnose – *C. capsici*; Fruits anthrachnose – *C. Gloeosporioides*

Witch’s broom: Abnormal proliferation of shoots. Originally given to the infection by the fungal that induces the production of shoots look-like broom.

Phyllody: The deformation of the flower due to either infection of disease or some other external factors causing the flower (petals) to be stunted or replaced by leaf like-structures.



leaf spot



leaf tipburn or scorch



leaf blister



distortion



scab



defoliation



vein enation



chlorosis



edema



wilt



vein clearing



tip dieback

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PATHOGEN SURVIVAL

The survival of a parasite between cropping seasons and its effective dispersal to uninfected plants are crucial aspects of the plant disease cycle. If either of these is prevented, the disease will not occur. Most pathogens possess mechanisms to survive intercrop periods or periods of unfavourable environmental conditions.

Many pathogens can **survive without** a susceptible host even under the most unfavourable conditions. Many plant diseases survive from one growing season to the next on plant debris, seeds, and alternate hosts or in soil. Because of pathogen survival, it is important to remove and properly dispose of any infected plant materials. It is also important for the farmer to know about the diseases that affect each crop throughout its growth period, as well as the conditions might be altered to prevent its spread.

Continuous infection chains

The survival of most plant pathogens requires the repeated infection of host plants. This is known **as the infection chain**. The infection chain can be **continuous** or **discontinuous** (incorporating a resting phase). **Continuous infection chains** can involve the same or alternative hosts. The parasite survives by continually infecting plants of the same host species, or, if the host species has a dormant or intercrop period, alternately infecting the main crop species and another, often related, species, the **alternative host**. If the alternative host does not display disease symptoms, it is called a **disease carrier**. The parasite does not form resting structures, and it is dependent on the presence of a susceptible host species. If the crop species grows throughout the year, for example, in tropical areas, the parasite can survive by continuously infecting new individuals of the same host species. Where the crop is not grown year-round, self-sown individuals of crop species that grow by the side of the road or as weeds in paddocks can act as hosts for parasites between cropping seasons.

There are some plant pathogens that cannot be transferred directly from one plant to another plant of the same species. They require another, completely unrelated, species to act as a **vector**. These vectors are usually, but not always, insects, and are referred to as **alternate hosts**. Unlike the case of alternative hosts, which are used opportunistically and where necessary, the alternate host is a necessary step in the infection cycle. If there is no alternate host available, even if there are susceptible plants available, the infection chain is broken.

Discontinuous infection chains

Discontinuous infection chains usually involve an epiphytic, saprophytic or resting phase. During an **epiphytic phase**, the pathogen survives on the surface of their host or other plants in a non-parasitic relationship as an epiphyte. Pathogens that go through a **saprophytic phase** survive during intercrop periods on diseased plant debris or other organic matter on or in the soil. Some of them can compete very effectively with the normal soil microflora. Others specifically inhabit

the diseased plant debris. Fungi and nematodes are able to form **resting structures** that enable them to survive long periods without a suitable host, or when environmental conditions are unfavourable.

The **resting spores** (*oospores*, *teliospores* or *chlamydospores*) of some fungi can survive for twenty years or more. Some of them are triggered to germinate only by secretions from the roots of suitable plants, reducing the risk of germinating without an available host. Other fungi produce **sclerotia**, which can also survive in the soil for periods ranging from months to years. Fungi can also produce sexual fruiting structures (such as *cleistothecia*, *perithecia* and *pseudothecia*) during the resting stage. Some fungi go through a resting stage after infection, called a **latent infection**. For example, loose smut fungus of wheat infects wheat embryos in the flowers, becomes dormant and is activated again when the seed germinates. When the plant matures, the fungus produces teliospores in the place of inflorescences. Nematode eggs can survive for long periods in egg cysts or gelatinous egg masses, which reduce the rate of egg desiccation.

PLANT DISEASE DIAGNOSIS

To correctly diagnose plant disease problems, a few basic steps must be followed.

1. Take a close-up view

The most obvious place to look first is up close.

Use a hand lens if necessary. Don't stop at the first or most obvious symptom; check for more. You want to find all of the symptoms. Look for symptoms on: leaves, stems, roots, flowers and, fruits. Cut open a branch or stem to look for vascular problems. Vascular problems show as discoloration of vascular tissue, leaf or stem wilting and sudden wilting of a section or a total plant.

2. General view of the field and environment

Stand back and look at the overall picture. Consider the total environment: weather, soil, stage of development for plant and pathogens, cultural practices and, condition of other plants in the area. A plant growing in the wrong location may be stressed. Consider pesticide applications, recent construction or digging, and weather conditions.

3. Establish cropping and management history

Determine when the symptoms became apparent. The onset of a problem may be due to a cultural practice, the seasonal appearance of a disease or insect, or a weather-related event. Remember that long-term stress is slow to appear, taking a year or more at times.

Is the problem spreading? This may indicate it is a pathogen. Are plants of other species affected? Diseases are usually species-specific. Problems caused by environmental factors do not spread, although the symptoms may become more severe.

4... Observe patterns and speed of spread

Symptoms caused by biotic agents usually are random in the field. Such symptoms also do not spread rapidly, that is, where one or a few plants are infected, it is unlikely that you will find the entire field covered with symptoms two or three days later.

5. Apply your knowledge of plant pathogens/diseases and consult repositories

You must know what the plant should look like to be able to determine abnormalities (Healthy Vs. Disease). Check the references to see what problems are typical for a particular species. Gather all the information you can to help you make the diagnosis. Remember that there is usually **no single cause**. There may be a **primary cause**; however, it may be associated with cultural or environmental conditions. Just as there is probably no single cause, there is usually no single symptom. **Search for all of the symptoms. Orderly thinking and good questions is the key to accurate diagnoses.** When in doubt about a diagnosis, turn to manuals for assistance or a second opinion.

6. Provide recommendations for control

Proper recommendation on action to be taken depends on the circumstances of each disease situation. The first decision to be made is whether any action should be taken at all. If the crop is within 3-4 days of harvesting, any control measures deployed may not be beneficial. In addition, applying chemicals to a crop that is about to be harvested for consumption would not be wise, as there will be chemical residue on the crop. If however it is decided that control measures can be done, the most effective, economically feasible, and environmentally friendly control method should be taken. Where only one or very few plants show symptoms of bacterial or fungal infection, that plant can be rouged out and destroyed, followed by close monitoring of the field to see whether the disease spreads. If the disease appears to be spreading fast, immediate application of chemical control may be necessary. Should chemical control be chosen, the correct chemical for the organism in question must be used. Not all fungicides will kill or deter the spread of all fungal species. Some fungicides may be effective against basidiomycetes but not against ascomycetes.

Abiotic agents in plant disease diagnosis

Nonliving or abiotic agents can indirectly result in plant problems. Additionally, several factors in the plant's environment can produce disease-like symptoms: weather extremes, high winds, high or low temperatures, nutrient deficiencies, physical damage and poor cultural conditions. Frost often damages buds and leaves in early spring. Hail can cause leaf spotting or holes. Drought and high winds result in wilting and in extreme cases, browning and curling. Air, water and soil pollution affect plant health and can produce disease-like symptoms. Soil imbalance, resulting from construction or other dumping, or misapplied garden chemicals can cause damage and disease-like symptoms. Dumping of household,

automotive and industrial chemicals can also produce plant damage. Plant disease can result from a combination of abiotic agents and biotic agents. Plants may be initially placed under stress by nonliving agents. This creates susceptibility in plants for attack by living agents. Drought may damage roots which then are more likely to be infected by fungal diseases.

Random or Uniform Patterns

Random distribution of symptoms on injured plants is usually caused by a biotic factor, such as infectious disease pathogens or an insect/animal. Uniform patterns are generally associated with abiotic or non-infectious agents like pesticides, fertilizers, environmental or site stress and mechanical damage.

Collecting Specimens

Plant specimens that are to be diagnosed should be taken from the area where symptoms are showing on living tissue. Dead plants are often invaded by secondary pathogens which may hide the original problem. **Collect several representative samples** showing various stages of disease development. A generous sampling will assist in diagnosis. If possible, collect the entire plant, including roots. Wrap the specimens in dry paper. Do not moisten them or seal them in plastic wrap or plastic bags. Never mix different specimens in a single bag. A fresh sample is required. Complete the diagnostic form as thoroughly as possible. This will result in better diagnosis.

Koch's Postulates

The fact that a particular organism is associated with the symptoms of a disease does not necessarily imply that the organism concerned caused the disease. To determine with certainty that a particular microorganism is the **cause of the disease** rather than some incidental contaminant, it is necessary to examine critically its relationship with the host plant. In 1884, Robert Koch provided the first experimental proof of disease causation (pathogenicity) by applying a set of rules –now known as Koch's postulates, which are universally accepted. These basic rules must be satisfied before any microorganism can be regarded as a pathogen. Though accepted, it is all too often over looked and there are diseases with putative causal agents that have never been demonstrated by Koch's Postulates. Whenever one speaks about, or hears about, a disease, one should always seek to find out if Koch's Postulates were performed.

The rules involve five steps outlined:

- a) The suspected pathogen must be consistently associated with the same symptoms
- b) The organism should be isolated in culture, away from host. This precludes the possibility that the disease may be due to malignant tissues or other disorders of the host itself.
- c) The organism should then be re-inoculated into a healthy plant (host) of the same species.
- d) Symptoms should then develop which are identical to those observed in the original outbreak of disease.
- e) The causal agent should be re-isolated from the test host into pure culture and should be shown to be identical with the microorganism initially isolated.

In general, Koch's postulates are only suitable for the diseases caused by the fungal and bacteria because these microorganisms can be cultured on artificial medium, and not for obligate parasites such as viral diseases and those caused by viroids. With these, inoculation on a healthy susceptible host under suitable conditions of isolation must be substituted.

Molecular tools for plant disease diagnosis (to be covered in more detail later)

The failure to adequately identify and detect plant pathogens using conventional, culture based morphological techniques has led to the development of nucleic acid based molecular approaches. These techniques are becoming more available that if used correctly would complement or replace time-consuming laboratory procedures. Others are still in developmental stages. Some procedures require laboratory equipment and training, while other procedures can be performed on site by a person with no specialized training

- a. Elisa diagnostic tests**
- b. Direct tissue blotting**
- c. Nucleic probes**
- d. Squash blot method**
- e. Polymerase chain reaction (PCR)**

ELISA Diagnostic Kits

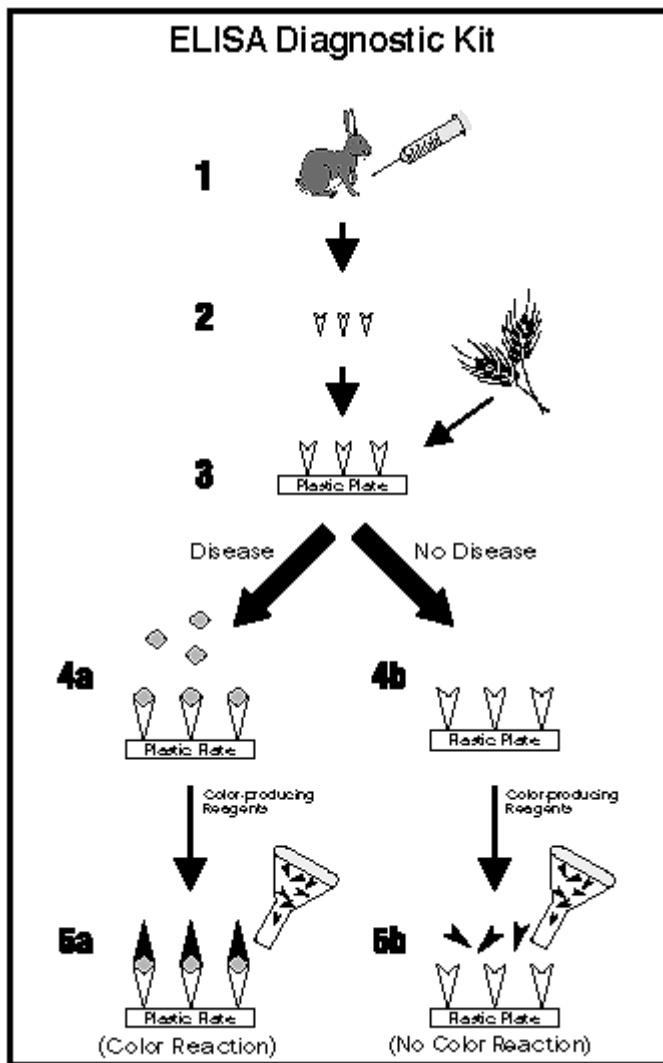


Figure 1.

A number of disease detection kits have been developed for use at the site where a disease is suspected. Some tests only take five minutes to perform.

The diagnostic kits are based on a method that uses proteins called antibodies to detect disease-causing organisms of plants (plant pathogens). The technique used is called ELISA (enzyme-linked immunosorbent assay). This assay is based on the ability of an antibody to recognize and bind to a specific antigen, a substance associated with a plant pathogen. The process is illustrated above. The antibodies used in the diagnostic kits are highly purified proteins produced by injecting a warm-blooded animal (like a rabbit) with an antigen associated with one particular plant disease (1). The animal reacts to the antigen and produces antibodies (2). The antibodies produced recognize and react only with the proteins associated with the causal agent of that plant disease.

These antibodies are bound to a plastic plate or similar detector unit in a test kit. The

individual running the test prepares the plant sample by grinding it between two pieces of abrasive paper. The ground plant sample is placed in a bottle filled with an extraction liquid. This liquid is then placed in wells in the plastic plate (**3**). If the disease-causing organism (pathogen) is present in the sample, the specific antibodies in the plastic plate will bind to pathogen-associated proteins and adhere to the unit (**4a**). A second antibody is added that also reacts with the pathogen-associated proteins. This antibody is special because it can react with color-producing chemicals called reagents (**5a**). Color changes on the unit's surface indicate a positive (disease present) reaction. If no pathogen-associated proteins are present (**4b**), the detector antibodies cannot bind to the color carrying reagents and are washed away (**5b**). At the present time, a limited number of these onsite disease detection kits are available. Many ELISA diagnostic kits are available for use in the laboratory, however, and can test for a wide range of plant pathogens. Examples of diseases ELISA kits can detect include bacterial canker of tomato, soybean root rot, and bacterial blight of geranium.

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Direct Tissue Blotting

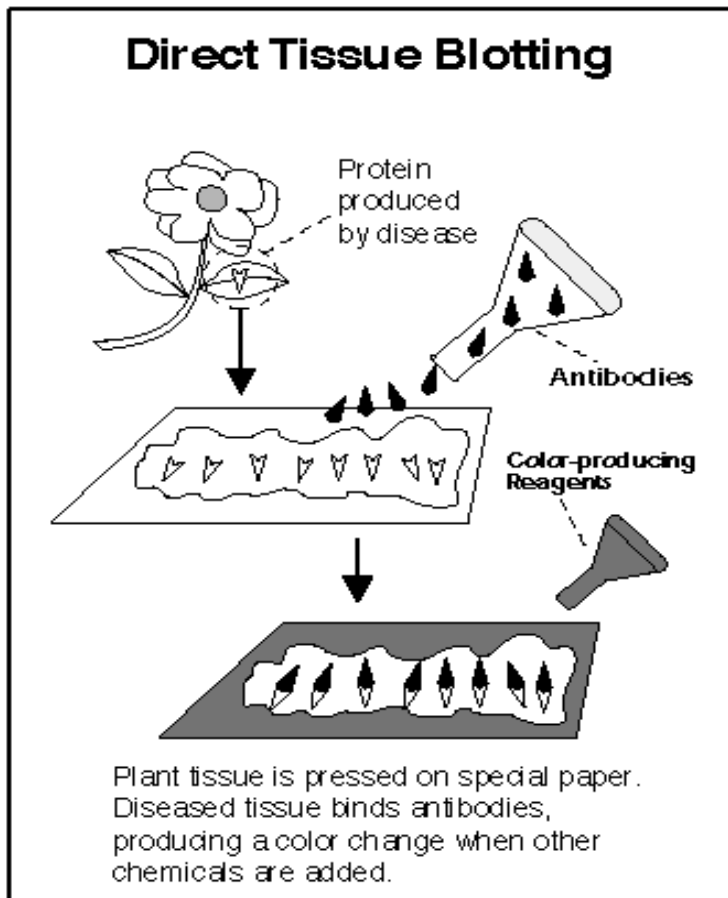


Figure 2.

Another diagnostic assay that also uses specific antibodies as a detection tool is known as **direct tissue blotting**. With this technique, the location of a disease-causing pathogen within the host plant can be determined, allowing earlier detection and a better understanding of how a disease progresses through a plant.

Host plant tissue is pressed onto a special piece of paper. Antibodies that bind only to the disease-causing pathogen in question are then introduced to this paper. A color change indicates a positive result and shows the location of the pathogen in the host tissue (See figure. above).

Nucleic Acid Probes

Another set of tools that can be used in plant disease diagnostics is **nucleic acid probes**. These probes are fragments of nucleic acid arranged in a sequence complementary to that of the **DNA or RNA of the disease-causing pathogen**. Because the sequences complement each other, the probes can be used to identify specific diseases.

Squash Blot Method

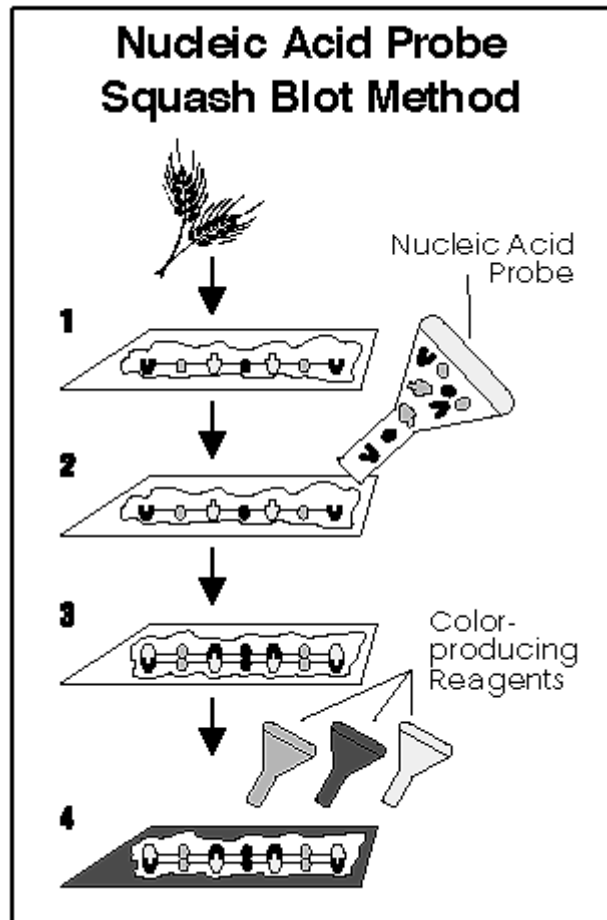


Figure 3.

A method that uses is known as the squash blot method (**figure 3**). This technology is similar to direct tissue blotting, in that it uses nucleic acid probe technology. Tissue from a plant that is suspected to be diseased is "squashed" onto a special piece of paper, called a membrane (1). This membrane is then treated with a probe (2) that can bind or hybridize with nucleic acid of the plant pathogen suspected to be in the plant tissue. Hybridization or binding will occur when like sequences are present (3). After adding several more substances to the membrane, a color reaction (4) indicates that the probe and pathogen nucleic acid sequences have hybridized and the disease is present. No color reaction means the test for the disease is negative.

Polymerase chain reaction (PCR)

Molecular diagnostic began to develop a real momentum after the introduction of polymerase chain reaction (PCR) in the mid 1980s and the first PCR based detection of a pathogen in diseased plants was published in the beginning of 1990s. To date an increasing number of diagnostic laboratories is adapting molecular methods for routine detection of pathogens. With the advances in molecular biology and biosystematics, the techniques available have evolved significantly in the last decade, and besides conventional PCR other

technologically advanced methodologies such as the second generation PCR known as the real time PCR and microarrays which allows unlimited multiplexing capability have the potential to bring pathogen detection to a new and improved level of efficiency and reliability. The specificity and sensitivity of detection of pathogens has greatly improved and pathogen detection is becoming simpler and faster in spite of the associated technical and economic challenges. **Diagnostic specificity** is defined as a measure of the degree to which the method is affected by non target components present in a sample, which may result in false positive responses. **Diagnostic sensitivity** is defined as a measure of the degree to detect the target pathogen in the sample, which may result in false negative responses.

A high degree of diagnostic accuracy is characterized by the ability to detect, true and precisely the target micro organism from a sample without interference from non target components. The specificity of PCR be it conventional or real-time depends upon the designing of **proper PCR primers** that are **unique to the target organism**. Highly conserved gene regions are often the target for designing primers. Closely related microbial species often differ in a single (**single-nucleotide polymorphism** (SNPs) to few bases in such genes. PCR allows detection of such SNPs. c detection of pathogens. PCR is a highly sensitive technology.

Nucleic acid based detection methods currently applied in pathogen detection are based on nucleic acid hybridization or PCR. These methods can be designed to detect either DNA or mRNA. Whereas DNA based detection method is often more straightforward than that of mRNA, the stability of DNA leads to the possibility that DNA based methods yield positive results from non-viable or dead pathogens. One of the main goals of pathogen detection system, besides determining the presence and absence of the pathogen, is the viability since in the event of positive result it is important to know whether the pathogen detected poses threat to crop production or food safety. The lack of discriminating viable from dead cells is a pitfall common to the nucleic acid based detection systems including microarrays and diagnostic PCR. In order to circumvent this problem many studies consider enrichment culturing (BIO PCR) instead of direct PCR. While the system allows the detection of only viable cells and helps in elimination of possible PCR inhibitors, it is not appropriate approach for quantitative assay. Therefore, the lack of ability to distinguish between viable and dead cells, and the lack of sample preparation methods that do not involve enrichment culturing are currently limiting the implementation of quantitative PCR for routine diagnostic use. Molecular methods for inferring pathogen viability focus on detecting mRNA in a sample as mRNA species are believed to be labile with a very short half life (seconds to minutes) after cell death.

For Pathogen detection and quantification, a second generation PCR known as real time PCR has become an emerging technique for the detection and quantification of micro

organisms in the environment. In PCR the target DNA sequence is amplified over a number of denaturation-annealing-extension cycles. Unlike in the conventional PCR, where only the final concentration of the amplicons may be monitored using a DNA binding fluorescent dye. In the quantitative real time PCR, the concentration of the amplicons is monitored throughout the amplification cycles using a group of fluorescent reagents. The fluorescence intensity emitted during this process reflects the amplicons concentration in real time. Undoubtedly most of the future tests will be quantitative in nature and the real time detection system will be a method of choice. The real time data will serve as useful basis for establishing inoculum threshold levels and detailed analysis of disease epidemics.

Crops can be infected by numerous pathogens and they may be present in plants in complexes. Therefore, it is desirable to develop technology that can detect multiple pathogens simultaneously. A multiplex PCR, a PCR variant which is designed to amplify multiple targets by using multiple primer sets in the same reaction, and real time PCR offer better multiplexing possibilities. However, multiplexing is still limited by the availability of dyes emitting fluorescence at different wavelengths.

The DNA microarray technology originally designed to study gene expression and generate single nucleotide polymorphism (SNP) profiles has emerged as the new pathogen diagnostic technology which in theory, offers a platform for unlimited multiplexing capability. The principle of microarray is the hybridization of fluorescently labelled sequences or targets to their complementary sequences spotted on solid surface, such as glass slides, serving as probes. Tens of thousands of such DNA probes can be spotted in a defined and addressable configuration on the glass slide forming the chip. The unlimited capability for simultaneous detection of pathogens makes microarrays to be an approach with a potential capacity of detecting all relevant pathogens of a specific crop. Development of microarrays for diagnostic applications is indeed a recent history.

In plant pathology the method was applied for identifying **oomycete, nematode bacterial and fungal DNA from pure cultures**. However, for application in practice, pathogens should be detected from environmental samples (plants, soil, etc.). Recently the possibilities of parallel detection of pathogens from such environments have been demonstrated. However some challenges still exist compared with studies using pure cultures, these must be addressed. The effort to add a quantitative aspect to microarrays must continue in addition to more work on addressing the challenges of working on environmental samples where contaminants (humic matter, organic substances, heavy metals etc) may interfere with DNA hybridization and affect the performance of microarrays. Adding innovative molecular tools for differentiating viable from non-viable organisms should be given emphasis in developing diagnostic assays.

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