**Plant Pathogens culture, diagnosis and detection low costs technique**

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**Abstract:**

Pathogenic microorganism produce plant diseases, which pose a severe danger to plant development, food sustainability, and natural habitats. Plant protection play an important role in agriculture for the food quality and quantity. The diagnosis of plant disease and the identification of the pathogens are essential pre-requisites for their understanding and control. Among the plant pest, the pathogens have devastating effects on plant productivity and yield. To attenuate or eliminate the effects of plant pathogen incursions, an adequate strategy for prior detection and speedy intervention is necessary. As a result, identification technologies are critical in plant wellbeing management, observation, and statistical pathogenic risk evaluation, leading to improve optimal strategies for mitigating and preventing microbial risks. Plant pathologists have shifted to more precise and efficient procedures, in order to minimize the time it takes to diagnose a disease. This study emphasizes on rapid and accurate approaches to recognize the existence of plant pathogens at a preliminary phase of pathogenicity until signs develop in the organism, as prevention is the optimal approach for protecting plants from diseases.

**Keyword:** Pathogen, Detection, Plant pathology techniques, Diagnosis

**Introduction:**

Various fungal and fungal-like infections have been developed, triggering disaster on forests and recreational tree varieties1. Pathogens, in particular, are harder to identify, particularly when plants are nonspecific at the point of aesthetic screening, and as a result, they are recognized at lower proportions than arthropods or nematodes2.

Plant diseases from different ecological zones can sometimes acclimatize to novel biotopes and act antagonistically on new-fangled host flora. They can also mutate, recombine, hybridize, and develop new viruses once they've been introduced to new territory4. Climate change, including expected increases in actual temperatures and amend in precipitation system, has an impact on pathogen behavior in recent years, and as a result, all of these changes will have a significant impact on host-parasite interactions at the tree, ecosystem, and countryside levels2. Foreign plant pathogens, in combination with weather alteration, may cause new ailment contagion, complicating efforts to control outburst. Biosecurity protocols for plant safeguard have been established to evade the spread of invasive infections and to aid in their elimination4.

In order to diminish the crash of persistent and native organisms in agriculture and forestry, a harmonised group of distinctive scientific, methodological, and conceptual solutions is needed to limit and/or prevent the entry and establishment of new diseases5. In this review, we present an impression of innovative, quick, spiky, and steadfast technologies capable of detecting woody plant infections before symptoms appear in the host, as prevention is the greatest strategy for protecting plants from diseases.

Seclusion of fungal infections from plants is commonly achieved by inserting tiny quantity of contaminated tissue on agarized growth media6. In multifarious natural surroundings, such as plant tissues, the pathogenic fungus forms a diverse marginal amidst a plethora of varied microorganisms that swiftly colonies the diseased host7. Morphological or genetic characteristics can be used to identify axenic cultures8. In the first scenario, light microscopy is used to examine the fungus's characteristic fruit bodies (conidia and spores) standard pathogen identification methods are time-consuming and demand special expertise from the operator9.

**Identification methods:**

* **ELISA**9, 10**-** A number of serological approaches, mostly based on the enzyme-linked immunosorbent test, have been developed to speed up the detection of plant diseases and consent to their detection in the field (ELISA). Using a monoclonal antibody tagged with fluorescent chemicals, these approaches are used to detect infections. The ELISA test includes the identification of an unambiguous analytic in a liquid sample and is simple to repeat.
* PCR (Polymerase Chain Reaction) technique.
* (RFLP) Restriction fragment length polymorphism.
* DNA-fingerprinting.
* Random amplification of polymorphic DNA.



**Fig.1 Identification techniques**

The biggest disadvantage is that this procedure necessitates the use of laboratory equipment. Portable immunoassay approaches were developed to circumvent these constraints12. (LFDs) Lateral flow devices are a simple paper-based dipstick test that can detect the presence or absence of a target analyte in a liquid sample without the use of specialist laboratory equipment5. This approach has gained popularity in recent years, enabling for quick identification of plant diseases in the field. This process is trouble-free to exploit and can perceive and categorize disease-causing substances in seconds13.

**Future technologies:**

These methods effectively 'piggyback' on publicly available platforms utilised in other areas, including as study, clinical diagnostics, and, more recently, native soil protection exposure. Its prevalent availability enables the creation of a business market for the precise reagents and consumables required, lowering prices and ensuring long-term viability8.

**Real-time PCR**15 **:**

These include nucleic acid sequence-based amplification (NASBA); helicase-reliant isothermal augmentation; and additional isothermal amplification approaches. PCR-based techniques- the exponential nucleic acid amplification reaction and rolling circle amplification methods all have the advantage of not requiring composite thermal cycling tools while retaining the specificity and sensitivity recompense of PCR-based methods.

**Diagnostic microarrays and direct nucleic acid sequencing:**

Microarrays have the potential to be a generic approach for detecting a wide number of known infections in a single test in the diagnostic laboratory16.

**de novo sequencing methods:**

Deep sequencing of cDNA and DNA amalgamate by means of general primer sets (e.g. 16S primers for bacteria) has been demonstrated to recognize the existence of pathogen sequence in recent14.

**Direct tuber testing:**

Taking eye cores from dormant tubers and cultivating them in a greenhouse for several weeks before analysing the sprouts produced is a standard method17.

This is used to assess the fraction of virus-infected tubers concealed in a particular seed stock by testing at least 100 tubers separately or in minute set, signifying its eligibility for grade at which it should be classed18.

Optical excitation and detection of amplified pathogen specific nucleic acids15, 19:

A pathogen detection system that uses optical excitation and detection of amplified pathogen specific nucleic acids to detect pathogens. To acquire the diagnosis, the device uses commercially available off-the-shelf (COTS) optical components, which are shown on a cell phone or palm-top computer. Loop-mediated isothermalamplification, an isothermal DNA amplification technique, is used to make the diagnosis (LAMP). LAMP works by heating a biological sample containing pathogens and certain chemicals.

DNA primers (needed for amplification), appropriate reaction enzymes, and other components (Bst DNA polymerase;dNTPs) for 30-60 minutes at a steady temperature of 65 degrees Celsius. Before the reaction, a DNA-binding fluorescent dye is introduced to the LAMP reaction mix. The primers amplify any pathogenic DNA that may be present in the sample by heating it. The virus of interest's specialized primers rapidly multiply (replicate) its DNA[2], while the negative sample stays un-amplified.

**Micropropagation**20**:**

Plant clonal propagation in closed vessels under aseptic conditions is known as micro propagation. The plants are cultivated in vitro, which means 'in glass,' inside the containers, on culture media that contains nutrients and growth regulators. Soil-grown plants, on the other hand, are referred to as in vivo. Plants propagated in vitro are smaller than plants propagated in vivo, hence the term "micro propagation." Rose varieties are clonally propagated and so make good micro propagation candidates.

**Autoclave**21 :

The medium, glassware, and instruments are sterilised in an autoclave, which is essentially a big but sophisticated pressure cooker. Commercially accessible autoclaves come in a variety of sizes. To disinfect media, water, and glasses, high-pressure heat is required. At 1210 C and 1.05kg/sq.cm (15 pounds per sq. inch) pressure, some fungus and bacteria spores are destroyed. Steam autoclaves that generate their own steam are more trustworthy and faster to use.

***Working***21***:***

It consists of a double-jacketed vertical cylinder composed of a strong metal that is separated by an iron sheet case. The lid is composed of gun metal and is held in place with buffer files screws. With the addition of an asbestos washer, it becomes airtight. Water is contained in the cylinder up to a specified level. The sterilised items are put on a perforated diaphragm that serves as a platform above the water level. A gas burner or an electric heater might be used to heat the room. The top of the chamber has a steam tap and a pressure gauge with a safety valve. The pressure used is 15 lb/inch for 15-20 minutes; at this pressure, H2O boils at 121°C. During operation, the air in the chamber must be supplied with saturated steam; otherwise, the temperature required will not be achieved. The temperature of steam, not the pressure, is what kills the organism. The length of time it takes to achieve sterility is determined by the nature of the material to be sterilized, the type of containers used, and the amount of the material to be sterilized. Autoclave sterilization kills all organisms, including spores. Protein coagulation causes the death of cells and spores.

**Laminar airflow chamber**20**:**

The clean filtered air provided by the laminar flow chambers allows cultures to be handled in a contamination-free environment. The culture transfer area houses the laminar-flow cabinets. In addition to laminar flow cabinets, some big laboratories incorporate sterile rooms.

***Design and Working***21, 22***:***

They come in a variety of sizes. A 1.2m X 1.2m X 7.1m work bench with glass on top and a sunmica top end to end, as well as a tiny sink and a door. They have germicidal ultraviolet light for internal sanitation and cool white fluorescent lamps for uniform illumination. They provided an enclosed space in which air circulated and dust and microbe screening filters were placed at a consistent rate over the working surface. These are designed to maintain positive pressure in the transfer area throughout the experiment's transit. For airborne pollutants, the surface of the transfer chamber is hand-sprayed with 70% ethanol, the door is gently closed, and UV is turned on for around 45 minutes for interior sterilization. The glowing tubes are turned on when the machine starts up. The door is open and the blower is turned on. This creates positive pressure within, preventing air from rushing in. It is recommended that the researcher wears a clean lab coat, a sterile cap, a face mask, and rubs his hands with ethanol before entering the transfer room.

***Temporary immersion bioreactors***22.23***:***

Standard semi-solid plant tissue culture vessels have a seal that allows for little contamination and limited gas exchange. The light, temperature, and gases in the conventional vessel are determined by the environment of the conventional growth chamber.

Temporary immersion bioreactors are bioreactors in which cultures are submerged in the medium for a predetermined amount of time at predetermined intervals. Because of their simplicity construction and functioning, they are appealing low-cost options. In a common design, two jars (plastic or glass) carry the liquid medium and the cultures, with one holding the liquid medium and the other holding the cultures. A system in which a single vessel with a reservoir on one side is automatically tilted at predefined intervals is another variant of temporary immersion bioreactors.

**Air-driven bioreactors**24**:**

Filtered air is pumped into the bottom of the vessel in air-driven bioreactors, which aerates and agitates the medium, raising and circulating the culture. Depending on their volume, these air-driven bioreactors are referred to as simple aeration or bubble column bioreactors. Air-driven bioreactor containers are frequently built of clear or translucent materials with fittings, such as autoclavable clear glass or plastic. Alternatively, they can be manufactured of translucent flexible plastic that is not autoclavable and sterilized with gamma radiation. Simple airlift or bubble column bioreactors are simple to operate and cost effective. Submerged bioreactors have the supreme benefit of being competent to be incorporated into mechanized or programmed micro propagation systems. The mores from the bioreactor is hard-pressed through a bio processor, and this distinguish, sizes, and distributes the culture to vessels for disseminate growth in mechanized tools. An operator sends the multiplied culture to a mechanical cutting device for separation and subsequently to a vessel for propagule development in the mechanized system.

**Conclusion:**

Conventional methods of plant pathogen isolation has been require a great cost and highly technical to run them properly. Those techniques requires several days for identification and as result the data may sometime vary with that of actual. To overcome the problems like, accurate identification of pathogens, low costs for labor as well as for equipment’s has been resolved by discovering the low costs equipment’s. These low costs equipment’s are not only easy to handle, but also gives accurate results within the desired time. Most of the low costs techniques described above requires a particular environmental condition to show up their performance. The rPCR, De novo, micro propagation are new in this field, as these requires some time but economically they are the first choice for pathogen detection and destruction. Moving forward, the another one is the autoclave, which is almost present in every laboratory. This mot only detect the pathogen and foreign substance but kill them or destroy them completely. Hence, these developing techniques provides pathogens detection and characterization in a cheaper, and elevated manner in a very less time with accurate data.

Even these cost effective methods are continuously improving, there is no such thing called perfect and so they require some modification. But, still these methods are highly recommended to use as because of their in expensive design, less requirements and accurate results.

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