Current Advancements in Fungal Plant Pathology Molecular Diagnostics

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ABSTRACT: ***Phytopathogenic fungal species can cause enormous losses in quantity and quality of crop yields and this is a major economic issue in the global agricultural sector. Precise and rapid detection and identification of plant infecting fungi are essential to facilitate effective management of disease. DNA-based methods have become popular methods for accurate plant disease diagnostics. Recent developments in standard and variant polymerase chain reaction (PCR) assays including nested, multiplex, quantitative, bio and magnetic-capture hybridization PCR techniques, post and isothermal amplification methods, DNA and RNA based probe development, and next-generation sequencing provide novel tools in molecular diagnostics in fungal detection and differentiation fields. These molecular based detection techniques are effective in detecting symptomatic and asymptomatic diseases of both culturable and unculturable fungal pathogens in sole and co-infections. Recent advancements in normal and variant polymerase chain reaction (PCR) assays, such as nested, multiplex, quantitative, bio and magnetic-capture hybridization PCR techniques, post and isothermal amplification techniques, DNA and RNA-based probe advancement, and next-generation sequencing, have resulted in novel molecular diagnostic techniques in the field of fungal identification and distinctions. In single and co-infections, these molecular based detection methods are efficient in identifying symptomatic and asymptomatic illnesses of both culturable and unculturable fungal pathogens.***

KEYWORDS: ***Diagnostics, Fungal, Infections, Pathogen, Phytopathogenic.***

1. INTRODUCTION

The global causing major loss or damage to crops and considerably reduced quality and quantity of commercial products by plant diseases include fungus, Bacteria, virus and nematode. These losses represent a significant yearly danger to world food production. In addition, pathogens may influence people's health, particularly when the pathogen generates toxins in or on consumer items, on the ground or in post-harvest storages. In the control of plant diseases, many methodologies, tactics and approaches are employed. The production of resistant species through reproducing plants, genetically modified plants, the use of agrochemicals and physical techniques, application. Plant breeding, genetically modified plants, the use of agrochemicals and physical techniques, the use of bio controlling agents, and excellent agronomic and horticultural practices are all examples of these. These methods have made a substantial contribution to the extraordinary increases in agricultural yield and quality over the last few generations [1].

The main biotic agents which create catastrophic conditions in crops are the fungal plant infections. Approximately 8,000 plant diseases are associated with fungal and oomycetes species. Pathogenic fungi attack plants either alone or in conjunction with other forms of phytopathogens at any phase from the seedling stage through the seed maturation stage under natural environmental circumstances. Anthracnose, blight, cancer, dampening, dieback, gall, leaf spot, powdery mildew, rust, root-dressing, scavenging, and wilting are the most frequent diseases of plant pathogens. In certain agricultural systems of economic importance, these illnesses can lead to considerable losses in output, amount and quality. These diseases can cause considerable yield, quality, and volume loses in a variety of agricultural systems across the world, affecting important economic agronomical, horticulture, floricultural, and ornamental, as well as forest plant species [2]. In order to guarantee food safety and safety, the growing world population needs a well-organized management control of plant disease in agriculture. An effective and efficient framework for early warning and rapid reaction is important to fighting plant pathogens. In the field of plant protection, diagnosis of fungal plant pathogen is important as it helps to improve crop vigour and health. Therefore, the administration of fungal illnesses calls for precise diagnoses of diseases based primarily on the identifying of causal agents. Furthermore, fungal plant diseases must be confirmed even if the exterior signs are used to diagnose these illnesses. Furthermore, even if the diagnosis of fungal plant diseases based on exterior symptoms has been established to a sufficient degree, it is necessary to confirm them. Furthermore, for disease diagnosis, a full list covering a known plant disease, its usual signs and symptoms, and its recognized probable phytopathogen for a specific host is required [3].

Several advancements in the field of fungal phytopathogenic diagnostics were accomplished. Conventional techniques for diagnosing fungal diseases have employed obvious symptoms following plant fungal infections, such propagules of fungi, conidies, sclerotia, or mycelial symptoms of fungal disorders after infection, on the exterior surfaces of flora. These are the cornerstones of the diagnosis of fungal diseases. The traditional procedures are widely employed, comprise isolation and cultivation, reinoculation, microscopic techniques and biochemical tests that are disadvantages since they are fatigueous. The antigen-antibody binding concept underpins immunological diagnostic techniques, however certain drawbacks have been identified, including limited sensitivity and affinity in tests, as well as possible contamination from pollutants [4]. Furthermore, because to the high inconsistency and phenotypic serological flexibility of fungi, identification of fungal plant diseases has been ineffective. As a result, it is critical to apply and develop innovative and effective diagnostic approaches to combat fungal plant disease. As a result, plant-fungal diagnostics has shifted to molecular methods that make pathogen detection and quantification easier. In fungal diagnostics, molecular tests can overcome the limitations of traditional and serological techniques.

Additionally, bioinformatics databases like GenBank in the National Center for Information on Biotechnology (NCBI), Nucleotide Sequence Database Collaboration at the EBI and MycoBank offer platforms to document mycological nomenclature innovations, store and recover nuclear sequence sequences of pest infection fungi that accelerate more quickly [5]. Fungal plant diseases are rapidly developing and posing a danger to the world economy. As a result, it's critical to detect and identify phytopathogenic fungus quickly and accurately. The goal of this study is to outline the different molecular methods used to diagnose fungal plant pathogens, as well as their benefits and disadvantages. It also looks at the molecular techniques that may be used to identify previously existing, emerging, and re-emerging plant infectious fungus in a variety of agricultural species [6].

1.1 History of Fungal Biological Control Applications:

From ancient times, human beings have been trying to enhance crop output and to limit the seriousness of crop diseases by changing growing techniques that lower both initial inoculum and infections. Many techniques have been used to manage infections using fungal antagonists to discover microbes and their interactions. Roberts (1874) revealed antagonistic activity between Penicillium glaucum and bacteria by microbes in cell media by developing the term microbial antagonism. By culturing soil with microbes considered to have antagonistic capability, Hartley (1921) undertook the first effort at direct biological control of plant diseases. To combat damping-off caused by Pythium debaryanum, he treated forest nursery soils with thirteen antagonistic fungi. Weindling documented Trichoderma lignorum's ability to suppress plant-pathogenic fungi through mycoparasitism, as well as the first time a recognized antimycotic-producing antagonist was used in biological control. Modern biotechnology advances have increased the possibility for fungal antagonists to be used against a wide spectrum of plant diseases. Over the last few decades, numerous studies and experiments have been conducted to find novel fungal BCAs and assess their efficacy under various environmental circumstances.[7].

1.2 Integrated Applications of BCAs with Synthetic Fungicides for the Control of Plant Fungal Pathogens:

In the development of agricultural systems for controlling plant conditions comprising post-harvest illnesses and to protect crop production and quality, synthetic fungicides comprising of inorganic or organic elements are popular to use, largely because of their comparatively low costs and easy application. The control by plant fungal pathogens includes chemical substances such as Captan, dithiocarbamates, thiabendazole (TBZ) and imazalil (IMZ). However, the extensive and uncontrolled use of synthetic insecticides for crop conservation and post-harvest food preservation has resulted in fungicide resistance as well as severe impacts on humans, livestock, and wildlife, resulted in massive negative ecological repercussions. With combined field and postharvest treatments, significant biocontrol of postharvest diseases of fruits and vegetables may be accomplished. Combining or integrating a BCA with a chemical fungicide or physical adjuvant, either concurrently or in rotation, should result in improved disease suppression, assuming that the biocontrol agent is suitable with the fungicides. [8].

1.3 Fungal Antagonists:

The potential for the applications of fungi biocontrol agents against plant diseases has risen considerably, since the fungi have a relatively high (sexual as well as sexual) reproduction rate, shorter development time and are targets specific. Moreover, in the disappearance of the host, their form of parasitism may persist in the environment and therefore sustainability is maintained. Many fungal species have strategies to effectively defend plants against plant pathogenic fungal infections (Figure 1)[9].

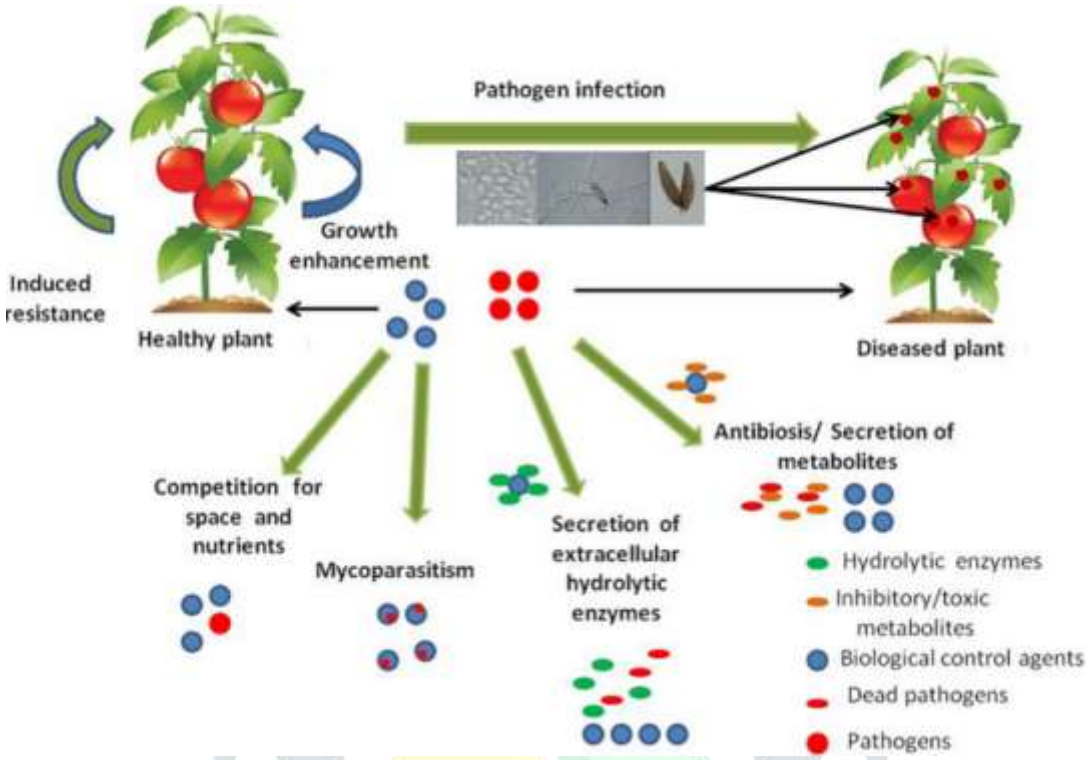

Figure 1: Key Mechanisms Of Action Involved In Biological Control Of Plant Fungal Diseases By Fungal Antagonists [9].

1.4 Molecular Tools for Fungal Detection:

Recognition and identification of molecular plant pathogens requires preanalytical processes such genomic isolation of DNA to lysis fungal cells effectively and recover the DNA, purification and quantification of the retrieved DNA. DNA must be recovered. A variety of DNA isolation methods are available from plant infection fungus. Recent techniques for the diagnosis of fungal plant pathogens employ commercial DNA extracting kits. The labs are dependent on conventional methods that include mycelia lyophilization, chitin cell wall interruptions, the grinding of DNA and the separation of proteins from the phenol-chloroform combination in sample buffer reagents, as well as precipitated with propanol. Pelleting, silica membrane, spin filter, and silica coated magnetic particle separation can all be used to purify the fungal DNA that has been extracted. Finally, a UV spectrophotometer may be used to measure the concentration of fungal DNA in the samples, which can then be diluted with ultrapure PCR grade water to get the desired DNA concentration.

- *End-Point PCR:* Advent of PCR changed the precise identification, especially fungus, of numerous plant diseases. In the PCR end-point, it permits reliable detection of plant diseases by developing either particular oligonucleotides targeting some fungal species or universal primers to multiply various pathogens and sequencing. In contrast with the ex-type cultures accessible in the NCBI GenBank database, a Basic Local Alignment Search Tool, can establish the identification of each isolated for each nuclear cycle of fungal isolates.
- *Nested PCR:* Nested PCR is a variant of end-point PCR that employs two sets of primer pairs for two rounds of PCR amplification to improve selectivity and specificity. Pilidiella granati causes pomegranate twig blight and crown rot, both of which are developing diseases in the pomegranate

industry. A nested PCR technique increased P. granati sensitivities and identification, allowing for the diagnosis of the causal agent even when the specimen included only 10 pg of P. granati DNA.

- *Multiplex PCR*: A multiplex PCR test employs a single reaction combination with numerous primer pairs to amplify numerous infections at the same time. Fusarium oxysporum, Bipolaris cactivora, Phytophthora nicotinae, and Phytophthora cactorum are pathogenic fungi that threaten the cactus industry's export sector. Multiplex PCR tests were used to overcome this challenge. The diagnostic tool was shown to be adequate for detecting and identifying these quarantine fungal infections in grafted cactus.
- *Quantitative PCR*: The QPCR allows the identification and measurement in real time in a PCR-Reaction Mix of certain DNA or RNA patterns of phytopathogenic fungi. Molecular qPCR diagnostics enabled the precision of the genome DNA to detect C. parasitica by the use of rDNA ITS sequences corresponding to one pathogen spore [10].

2. LITERATURE REVIEW

Elaine Ward et al. review some of the diagnostic tools currently used for fungal plant pathogens and describe some novel applications. Technological developments in PCR-based techniques such as PCR in real time provide quick, precise quantitative determination and are increasingly used to address practical challenges. PCR-based methods are used. A number of infections in wheat were simulationally detected and the fungicide tolerance in wheat pathogens was studied by molecular techniques. Data from such study may be utilized to enhance the management of diseases by logical choices and usage of fungicides and resistant crops. PCR based approaches can offer new tools that are more accurate and quicker than current methods for monitoring the exposures of a crop to pathogenic inoculum [11].

Bart Lievens and Bart P. H. J. Thomma review the most important recent advances in molecular plant pathogen diagnostics, with special attention to fungal molecular diagnostics. The diagnostic laboratories have been more and more engaged in rapid routine procedures that ensure that plant pathogens are reliably recognizable, sensitive and accurate quantified. Moreover, given that several diseases can infect plants or portions thereof, multiplex assays that can simultaneously recognize and quantify various pathogens are extremely desired. Technologies which can satisfy these criteria are being developed and applied in horticultural and agricultural practices, in particular those with the polymerase chain reaction. The DNA array method is now the most appropriate tool for plant pathogens multiplex identification. For multiplex detection of plant diseases, DNA array technology is presently the most appropriate approach. DNA arrays have recently gained a quantitative component, making them extremely appealing for a variety of academic and practical applications [12].

Leonardo Schena et al. studied and detected the phytopathogenic and antagonistic fungi by real time PCR technology. For the application of this method in plant pathology, four key chemicals are being employed. These chemistries may be classified in non-specific amplicon sequencing and sequence techniques. Non-specific Amplicon sequence techniques are based on the employment of a dye that transfers fluorescent light to double-stranded DNA. The fluorectant signal removes the need for processing steps after amplification, including gel electrophoresis and bromide dyeing with ethidium. This decreases considerably the time and effort necessary for analysis and enhances considerably the performance of PCR testing in an automatic diagnostic system and is thus suited for large-scale assessment. Real-time PCR allows for the precise, dependable, and high-throughput quantification of intended fungal DNA in a variety of environmental specimens, such as host tissues, soil, water, and air, allowing for new research positions in diagnosis, inoculum threshold levels, epidemiology, and host - parasite interrelations [13].

3. DISCUSSION

Plant pathogen generates worldwide serious losses or damages to crops and reduces the quality and amount of agricultural goods substantially. Worldwide trends are changing to reduce the use of chemical pesticides while many biological control methods, tactics and strategies are employed to manage plants. The management of infections and illnesses by fungal antagonists is crucial, and they are utilized worldwide as Biocontrol Agents (BCAs). The study also includes molecular techniques for fungal infection detection in plants. The molecular

techniques discussed in this study are precise, efficient, laboratory-based and sophisticated. To detect fungal plant diseases, the molecular techniques discussed in this study are accurate, effective, lab-oriented, and need sophisticated tools. Expertise in mycology and bioinformatics, on the other hand, is required to avoid misunderstanding of the outcomes of molecular biological studies. By integrating molecular approaches with other emerging technology breakthroughs for fungal disease detection, molecular approaches should become a site of care testing (POCT). Scientists are faced with the task of developing effective molecular diagnostics for agricultural diseases.

4. CONCLUSION

In recent molecular biological technologies, new, developing, already documented and recurrent fungal plant diseases have been detected and diagnosed more effectively. The diagnostics of the detection and diagnosis of phytofungal diseases are famous for conventional polymerase chain reaction (PCR) and variant assays, isothermal and post amplified tools, hybridization methods, and approaches for next generation technology. These molecular methods have effectively detected and treated symptomatic or asymptomatic illnesses in single, agricultural, horticultural, flora, ornamental and forest plain fields of cultivable and incultural pathogenic fungi. In solo and co-infections of agriculturally significant field, horticultural, floricultural, ornamental, and forest plant taxa, these molecular-based methods have effectively detected and identified symptoms and asymptomatic illnesses of culturable and unculturable fungal pathogens. Quantitative PCR has been widely utilized in the enumeration and separation of causative agents when the specimen load is too little to detect using different PCR-based techniques.

Currently, loop-mediated amplification (LAMP) is being used to identify Alternaria spp., Colletotrichum spp., Fusarium spp., Verticillium spp., and Puccin spp. in the field of fungal disease detection. Presently, loop-mediated amplification (LAMP) is exhibiting promise in the field of fungal disease identification, allowing for the recognition of Alternaria spp., Colletotrichum spp., Fusarium spp., Verticillium spp., Puccinia spp., Botrytis spp., and other pathogens that cause a variety of plant diseases. NGS may be used to discover novel and emerging diseases since it employs multiple platforms to sequence fungus genomes with no previous knowledge of the pathogen's sequencing.

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