

Advanced Techniques of Plant Pathogen Diagnostics

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Editorial

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DESCRIPTION

A multitude of PCR based diagnostic techniques are available in modern plant pathology. A researcher should have a concept of the target sequences that could be used in a DNA assay to detect and identify a plant pathogen. As a result, the data generated by phytopathogen genome sequencing must be accessible. Because the primers ensure the specificity of PCR, they must be chosen and designed correctly for any PCR analysis to be successful. The selection of primers is the initial step in PCR diagnosis.

Because the genomes of viruses and viroids are often small, comprehensive sequencing data and relevant primers are readily available in databases. The genomes of bacteria, oomycetes, and fungi have gained less information, despite the fact that the amount of data is expanding. General approaches to selecting specific known DNA target pieces are available for these diseases, and procedures based on screening random sections of DNA have been developed.

The DNA encoding ribosomal RNA (rDNA) is commonly employed as a target sequence in bacteria, oomycetes, and fungi. rDNA is suitable for diagnostic purposes due to a number of factors. Each cell contains many copies of rDNA, which improves detection sensitivity. The genes are found in all organisms and have a highly conserved 5.8 S regions, allowing rDNA to be used universally. At the same time, other sections, like as the Internal Transcribed Spacer (ITS) region, are very variables.

The conserved sections can be utilized to create universal primers for group identification of microorganisms within a taxon (for all oomycetes, fungi, or bacteria), whilst the existence of variable regions allows for race, strain, and isolate differentiation. Beta tubulin genes, which are linked to fungicide resistance, are another target sequence employed for fungal detection. The target pieces are usually derived from DNA found in bacterial plasmids and pathogenicity associated genes.

Random Amplified Polymorphic DNA PCR (RAPD-PCR), also known as arbitrary primed polymerase chain reaction, is one of the procedures utilized when the target nucleotide sequence is uncertain (AP-PCR). The RAPD-PCR is commonly used to study DNA polymorphism, gene mapping, and demographic and evolutionary biology, either alone or in combination with RFLP. The RAPD-PCR is significant for plant pathogen diagnostics since it allows for the screening and differentiation of sequences specific for closely related species, strains, races, and isolates.

RAPD-PCR uses annealing of single primers, as opposed to the described PCR analyses, which use two primers to restrict the generated sequence. After amplification, the primer binds to the genomic DNA's random complementary sequences, yielding a RAPD-PCR product of variable length that is partially or wholly homologous to the arbitrarily

primed sequence at both ends. The DNA polymorphism, which is caused by insertions, deletions, and base substitutions, affects the RAPD-PCR product, which shows up as the presence or lack of bands in the gel following RAPD-PCR. This approach can be used to amplify gene products from a variety of organisms, and the pattern of the bands after electrophoresis will be unique to each creature. To find a band that is specific for a target, many different primers must be examined. Highly specific primers can be made using specific bands.

Finally, it's worth noting that PCR isn't the only method for detecting amplification. The ligase chain reaction, for example, has been used to detect some plant diseases (LCR). It is based on the ability of a DNA-dependent DNA-ligase to ligate a DNA strand when the phosphodiesteric bond is ruptured in the presence of Adenosine Triphosphate (ATP) and Mg^{2+} ions. High specific activity in ligation of single stranded ruptures at the template, which forms the second complementary strand, and low specific activity in simultaneous ligation of two ruptures in both strands or rupture in single stranded DNA are two characteristics of DNA ligase work.

Finding two sets of primers complementary to each other and to the initially chosen fragment of the matrix (for example, DNA of some causative agent) in a "head to tail" arrangement from the 5' to 3' end are required for LCR implementation. The reaction mix accumulates the result, which is a ligated double-stranded DNA fragment that is structurally identical to the four primers employed, as soon as the second LCR cycle is completed. It is typical that even a single nucleotide mistake in the annealing location results in a negative result.

As a result, LCR has promise for improving plant pathogen identification and identifying point mutations in wild types of causal agents. The LCR has been modified to detect potato viruses A and Y in tubers, identify *Erwinia stewartii*, and distinguish *Phytophthora infestans*, *P. mirabilis*, and *P. phaseoli* from other *Phytophthora* species in a PCR format. LCR was paired with ELISA in the latter example.