# Introduction to Molecular Plant Pathology

**Molecular pathology** is an emerging discipline within pathology which is focused in the study and diagnosis of disease through the examination of molecules within organs or tissues. Molecular pathology shares some aspects of practice with both anatomic pathology and clinical pathology, molecular biology, biochemistry, proteomics and genetics, and is sometimes considered a "crossover" discipline. It is multi-disciplinary in nature and focuses mainly on the sub-microscopic aspects of disease. A key consideration is that more accurate diagnosis is possible when the diagnosis is based on both the morphologic changes in tissues (traditional anatomic pathology) and on molecular testing.

**Molecular Tools/Methods used for detection of plant pathogens**

- PCR
- Real-time PCR
- ELISA
- DNA Fingerprinting
- Molecular Marker
- Molecular/ Nucleic Acid Hybridization etc**.**

**Polymerase Chain Reaction (PCR)**: The polymerase chain reaction (PCR) is the most important and sensitive technique presently available for the detection of plant pathogens. PCR allows the amplification of millions of copies of specific DNA sequences by repeated cycles of denaturation, polymerization and elongation at different temperatures using specific oligonucleotides (primers), deoxyribonucleotide triphosphates (dNTPs) and a thermostable Taq DNA polymerase in the adequate buffer (Mullis & Faloona, 1987).

**Real-time PCR**: Real-time PCR is currently considered the gold standard method for detection of plant pathogens. This technique allows the monitoring of the reaction during the amplification process by the use of a fluorescent signal that increases proportionally to the number of amplicons generated and to the number of targets present in the sample (Wittwer *et al*., 1997). Many are the advantages of real time PCR over conventional PCR, including that this system does not require the use of post PCR processing (electrophoresis, colorimetric reaction or hybridization), avoiding the risk of carryover contamination and reducing assay labour and material costs.

**DNA Fingerprinting:** Fingerprinting approaches allow the screening of random regions of the fungal genome for identifying species-specific sequences when conserved genes have not enough variation to successfully identify species (Mc Cartney *et al.,* 2003). Fingerprinting analyses are generally used to study the phylogenetic structure of fungal populations. However, these techniques have been also useful for identifying specific sequences used for the detection of fungi at very low taxonomic level, and even for differentiate strains of the same species with different host range, virulence etc.

**A molecular marker** (identified as genetic marker) is a fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA. A genetic marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species.

**Classification of molecular marker**

1. Non PCR based molecular markers: e.g. R.F.L.P etc.
2. PCR based molecular markers: R.A.P. D & S.N.P etc.

**Types of molecular markers used in plant pathogens diagnosis and management**

1. Restriction Fragment Length Polymorphism (RFLP)
2. Random Amplified Polymorphic DNA (RAPD)
3. Amplified Fragment Length Polymorphism (AFLP)
4. Simple Sequence Repeats (SSR)
5. Single Nucleotide Polymorphism (SNP) etc.

**Future Perceptions:**

Information resulting from detection by improved molecular methods could be used to optimize disease control through more rational decisions about the choice and use of control measures. Besides optimization of PCR and real-time PCR protocols, the advances in microarray, microchip or biochip technology will allow to test simultaneously, the prospect of a wide variety of pathogenic microorganisms, and the potential of this tool will open new fields of studies in plant pathology.