

Chapter 4

Detection and Diagnosis of Important Soil-Borne Diseases: An Overview



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Abstract Soil borne pathogens are major group of phytopathogen causing numerous soil-borne diseases. Due to their persistent behaviour, huge losses in yield have been reported. Thus, to build an effective and precise management approach, these soil-borne diseases must be detected early, quickly, and accurately. The most common methods for identifying plant diseases in the past were basically based on morphological approaches and such approaches are highly time-consuming and lab or intensive. Molecular detection techniques could address these issues with greater precision and dependability. Collection of information regarding pathogen presence through molecular approach assist in taking timely decisions for early-stage treatments and pre-plant evaluation of the fields. Nowadays, polymerase chain reaction along with high-throughput sequencing methods provides a best window to check the soil health status, in which specific conserved region present in the microbes (16S and ITS) are amplified and sequenced. However, the effect of environmental condition on dynamics of phytopathogens could be exploited to develop prediction model, which allow anticipating the attack of soil borne pathogen prior to disease establishment.

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4.1 Introduction

The plant kingdom is recorded to have been infected by about 80,000 diseases, of which the soil-borne diseases occupy a majority of stake. It is more challenging to control soil-borne diseases as their diagnosis is difficult during the early stage of infection because the host is symptomless and has a long latent period (DeShields et al. 2018; Tarafdard et al. 2019). The diseases from soil-borne phytopathogens are key constraints in limiting the production and productivity of crops. Many of the soil-borne phytopathogens like *Fusarium*, *Rhizoctonia*, *Pythium*, *Phytophthora*, *Verticillium*, *Sclerotinia*, etc., cause yield loss in cereal, pulse, vegetable, fruit, and ornamental crops to the tune of 50–75% (Mihajlovic et al. 2017; Baysal-Gurel and Kabir 2018). The phytopathogens inhabiting soil are a diverse group of microbes from lower fungi to higher fungi to bacteria to viruses to even nematodes. However, all the members of this group share some basic features, which enable them to survive and live their part of life in the soil (Ghosh et al. 2019). Some of these phytopathogens form specialized survival structures like resting spore or melanized hyphae that are capable of surviving in the soil for a long period of time (Baysal-Gurel and Kabir 2018). Major diseases caused by soil-borne phytopathogens include rots (root rot, collar rot, stem rot, and head rot), wilts, blights, and damping-offs.

4.2 Important Soil-Borne Phytopathogens

In a list of top ten fungal phytopathogens of economic and scientific importance published by Dean et al. (2012), two genera namely *Botrytis* and *Fusarium* are soil-borne fungi. The species of these two genera cause massive losses in many agricultural and horticultural crops. In addition to these, *Verticillium*, *Phytophthora*, *Plasmodiaophora*, and *Sclerotinia* are also soil-borne fungal phytopathogens that are of economic importance. Among the soil-borne diseases of crop plants, rots are majorly caused by species of *Phytophthora*, *Pythium*, *Rhizoctonia*, *Aphanomyces*, etc., which have lowered the production of many crops very significantly (Clarkson et al. 2015). Additionally, wilt, yellowing, dieback, stunting, damping-off, root blackening, and cracking are other common diseases caused by soil-borne phytopathogens (Ghosh et al. 2019; Panth et al. 2020). Apart from the below-ground infections, some of these phytopathogens, namely species of *Sclerotinia*, cause infections at and aboveground levels in the form of collar and stem rots. The prevalence of soil-borne fungal phytopathogens spread from the Southern to North-west pacific.

Even with the abundance of fungal genera in the group of soil-borne phytopathogens, many bacterial phytopathogens are also an eminent part. These soil-borne

phytopathogenic bacterial genera include *Erwinia*, *Streptomyces*, *Rhizomonas*, etc., and are responsible for causing scabs and soft rots. The viral diseases of soil-borne nature are rare as they have a necessity of living host, but many of them are carried by fungi and nematodes dwelling in soil or flow with the thin film of water around soil particles (Ghosh et al. 2019). Nematodes also constitute the soil-borne phytopathogen group and are responsible for about 10% annual global loss of agricultural production, amounting to over \$125 billion each year (Chitwood 2003). With the increasing temperature of the world, it is now suggested that the infection by soil-borne phytopathogens might increase as the reservoir of inoculum increases (Egidi et al. 2019).

4.3 Detection and Diagnosis

For a very long time period, the identification of plant diseases has been carried out by the experts on the basis of their knowledge and experience after viewing the affected plants through their naked eyes. Since the process of infection is influenced by various parameters consisting of inoculum type, growth stage of plants, and weather, it has become a tedious and exhaustive process for the experts to identify the disease. This process of disease identification has now become time consuming and expensive, so modern techniques are being utilized for the same (Mishra et al. 2020). The different methods utilized in the identification of soil-borne phytopathogens are mentioned in Fig. 4.1.

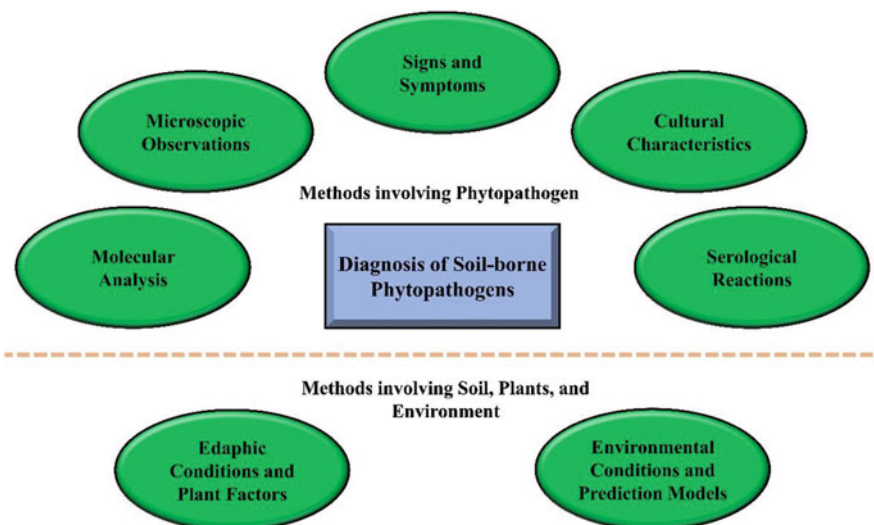


Fig. 4.1 Different methods utilized in the identification of soil-borne phytopathogens

4.3.1 Identification Through Visual Symptoms

The initial step of plant disease diagnostics is the identification of the phytopathogen (Riley et al. 2002). Whenever there is an infection in crop plants from soil-borne pathogens, there is a deviation from the normal functioning of the plants, which helps in predicting, confirming, and managing plant diseases prior to loss. The diagnosis of soil-borne diseases can be challenging, and it usually depends on a combination of observable symptoms and prior knowledge of common diseases that may be prevalent in your region. By comparing infected and healthy plant samples in the lab, a particular phytopathogen can be identified. The parts of phytopathogens that become visible on plants after infection are called signs and are more reliable than symptoms in the identification process. A hand lens and a knife are sufficient to diagnose disease in the field initially. Study of symptoms serves as additional support in the process of phytopathogen identification. Wilting of foliage, tissue discoloration, root decay, loss of vigor, stunted growth, distortion of tissues, and sudden death are some of the major symptoms in crops when they get infected by soil-borne diseases. These symptoms can be differentiated from the abiotic stress symptoms if the chlorosis, stunting, and deformation spread to the whole plant. As discussed earlier, the symptoms due to the soil-borne pathogen can be identified by the presence of physical signs of the phytopathogens, i.e., mycelial growth in the case of fungi, ooze in case of bacteria and cysts in case of nematodes. The signs and symptoms can help in distinguishing between the phytopathogenic causes and even predicting them up to the genus level (Table 4.1).

4.3.2 Identification Through Cultural Characteristics

When a particular phytopathogen of soil-borne origin is isolated and grown under lab conditions on different media, it shows certain characteristic features, which are utilized as one of the tools for identification. Different soil-borne phytopathogens grow and multiply on different media depending on their nutritional requirements. Some of the selective, semi-selective, and nonselective media for the growth of soil-borne fungi are PARP medium (selective for *Pythium* spp.), Mathur's medium (semi-selective medium having iprodione for *Colletotrichum* spp.), Czapek dox agar medium (semi-selective medium for *Verticillium* spp.), and potato dextrose agar medium (non-selective medium for *Rhizoctonia* spp., *Macrophomina* spp., *Fusarium* spp., etc.). Type of spore(s), its morphology, and mycelial structure are helpful in identification. Pigmentation is yet another cultural characteristic that is utilized for the identification of a particular phytopathogen. Development of orange-colored pigmentation with single-celled conidia confirms the suspected pathogen as *Colletotrichum* spp. Furthermore, if the conidia are acute on both ends, the species is *Colletotrichum acutatum* and if they are round, the species is *Colletotrichum gloeosporioides* (Freeman and Katan 1997). *Phytophthora* spp. have coenocytic

Table 4.1 Signs and symptoms of major soil-borne diseases

Signs and symptoms	Disease	phytopathogen (s)
Small elliptical, water-soaked lesion on root, poor stands, wilting, death of emerged seedlings, and whitish mycelial growth on the stem at soil level	Damping off	<i>Pythium</i> spp. <i>Rhizoctonia</i> spp.
Dark-brown sunken lesion near the crown and on leaves near the ground	Crown rot, crater spot, bottom rot	<i>Rhizoctonia</i> spp.
Soft, sunken dull orange lesion, accompanied with ooze and bad odor	Bacterial soft rot	<i>Erwinia</i> spp.
Soft and watery rotting of lower stem with profuse mycelial growth and formation of black sclerotia	Cottony rot, pink rot, white mold	<i>Sclerotinia</i> spp.
Thinning of crown, dieback, chlorosis of leaves, and decaying of roots and inner tissues at the base of stem	Root rot	<i>Armillaria</i> spp.
Stunting, wilting, darkened roots, and collapsing of plants	Phytophthora root rot	<i>Phytophthora</i> spp.
Stunted growth, leaf yellowing, and brown discoloration of vascular tissue	Fusarium wilt	<i>Fusarium</i> spp.
Stunted growth, leaf yellowing, and black discoloration of vascular tissue	Verticillium wilt	<i>Verticillium</i> spp.
Profuse gumming on stem from small and black lesions	Charcoal rot	<i>Macrophomina</i> spp.
Stunting and yellowing of plants, formation of galls, and distortion of roots	Root knot nematode	<i>Meloidogyne</i> spp.
Uneven root growth with tiny white to brown cysts	Cyst nematode	<i>Heterodera</i> spp.
Tan discoloration and rot of stem, bulbs, and basal plates	Stem and bulb nematode	<i>Ditylenchus</i> spp.

Source: Harrington et al. (1992), Koike et al. (2003)

mycelium with double-celled oospore and lemon-shaped conidia (Meszka and Michalecka 2016). The presence of white septate mycelium with branching at 45° and 90° angles and formation of brown to black sclerotia confirms the phytopathogen to be *Rhizoctonia* spp. Formation of melanized dark-brown microsclerotia at 10–14 days after inoculation confirms the phytopathogen to be *Verticillium* spp. (Zveibil and Freeman 2005).

Soil-borne bacterial phytopathogens grow in general on nutrient agar medium, King's B medium, and yeast extract mannitol agar medium. Colonies of bacteria are white or clear, transparent or opaque, smooth or rough, shiny, and mucoid-type. Some of the bacterial phytopathogens also produce pigmentation from cream to pale yellow to light pink (Khedr et al. 2014). *Rhizomonas* spp. produce opaque, sticky, and yellowish circular colonies on nutrient agar medium, which confirms their presence. *Streptomyces* spp. can be identified through their mycelial-type colonial growth on yeast extract malt extract medium (Shepherd et al. 2010).

4.3.3 Identification Through Microscopic Observations

Soil-borne phytopathogens can be very easily identified to genus level through microscopic observations. The differentiation between fungi and bacteria can be very well achieved through cultural characteristics. Thereafter, fungi can be identified through microscopic observations of their mycelium and spores, as knowledge about the spore shape, size, color, and arrangement is sufficient for predicting their taxonomic position. On the other hand, most of the bacterial phytopathogens are Gram-negative rods; hence, they can be identified by observing their shape and color after Gram's staining. The only Gram-positive bacterial group that are phytopathogenic are *Actinomycetes* spp. Furthermore, the Gram-negative ones can be identified by observing the presence/absence, number, and position of flagella. For example, *Erwinia* spp., which are responsible for soft rot, have a peritrichous flagellar arrangement.

4.3.4 Identification Through Serological Reactions

In serological detection techniques, unique antibodies react to phytopathogen-specific protein(s), giving a positive or negative result. Different soil-borne phytopathogens have varied reactions to different antibodies, and hence, a combination of antibody reactions is devised to form a serological study for identification. Most of the bacterial phytopathogens produce antibodies or related compounds, which are exploited in the serological assay test for their diagnosis. Most common serological methods used in the diagnosis of soil-borne phytopathogens are enzyme-linked immunosorbent assay (ELISA), tissue-blot immunoassay (TIBA), and quartz crystal immunoassay. For example, the resting spores of *Plasmodiophora brassicae* are detected through the use of highly specific monoclonal antibodies in indirect enzyme-linked immunosorbent assay and indirect immunofluorescence assay (Wallenhammar et al. 2012). *Rhizoctonia solani* are identified by using IgM monoclonal antibody in an LFD-based assay (Thornton 2008). One advantage of using the serological methods of phytopathogen identification is that through these methods we can diagnose them even at a very low detection limit (Lopez et al. 2003).

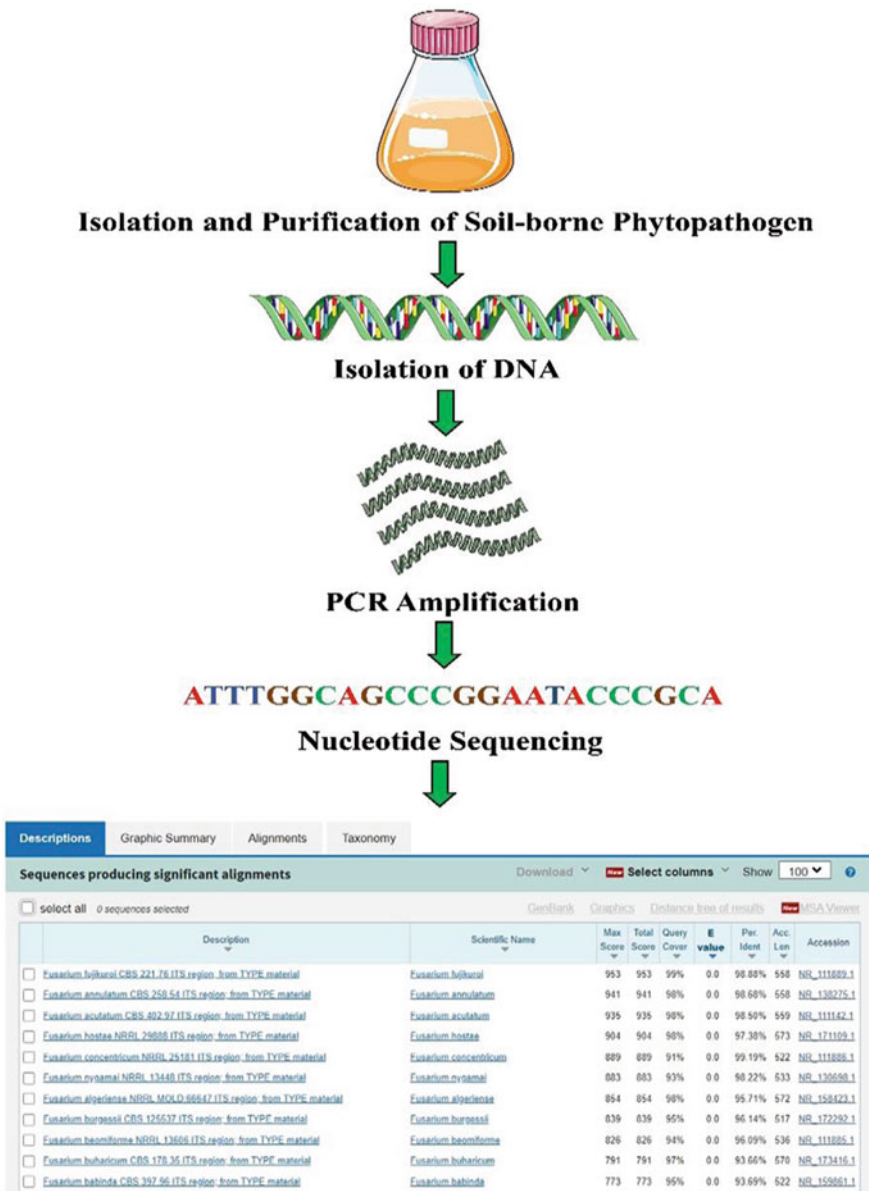
4.3.5 Identification Through Molecular Methods

Use of molecular techniques in the diagnosis of soil-borne phytopathogens has increased over the years. These techniques offer us an option of determining the phytopathogen at the species level with the help of a specific primer with a high level of sensitivity and precision (Zveibil and Freeman 2009). Polymerase chain reaction (PCR) is an economical and powerful tool that amplifies small segments of DNA or

RNA for identification. The working principle behind this technique is the hybridization of nucleic acid with complementary bases in recurrent cycles. The identification of phytopathogens through conventional approaches is a time-consuming process and requires proficient knowledge of physiology and taxonomy of the phytopathogen (Leslie et al. 2006; Thokala et al. 2015). In this method of identification, the conserved genomic regions (generally ITS in the case of fungi and 16S rRNA gene in the case of bacteria) of phytopathogens are amplified and sequenced. After sequencing, the obtained nucleotide sequence is aligned to the database sequences (Extaxon or Blast), thereby giving the name of identical organisms (Chittem et al. 2015). The process of identification through molecular methods is described in Fig. 4.2. The advent of real-time PCR has now provided the option of detection and quantification of soil-borne phytopathogens on a real-time basis. Various soil-borne phytopathogenic fungi such as *Colletotrichum michiganensis*, *Rhizoctonia solanacearum*, *Fusarium oxysporum*, *Alternaria* spp., *Phytophthora* spp., etc., are now commonly diagnosed by the scientific community using the PCR method. It can also be used additionally to study the genetic diversity within the species of soil-borne phytopathogenic fungi (Steimel et al. 2004; Reznikov et al. 2018). Rapid development in PCR has now enabled on-site detection of soil-borne phytopathogens (Ghosh et al. 2019). High-throughput sequencing methods are now additionally exploited with PCR for the diagnosis of soil health (Yuan et al. 2020).

4.3.6 Identification Through Analysis of Edaphic and Plant Factors

Different edaphic factors consisting of soil pH, temperature, nutrition, moisture, etc., are universally recognized as crucial factors in the development and spread of soil-borne diseases. These edaphic factors often affect soil micro-flora and fauna by regulating their production and diversity (Rajakaruna et al. 2008). When it comes to soil-borne phytopathogens, physical, chemical, and biological soil properties play a far more important role in defining their population and diversity (Nielsen et al. 2010). Thus, sampling of soil followed by phytopathogen-specific testing gives us an initial idea for moving forward with the diagnostics (Clarkson et al. 2015). There are different proposed soil sampling methods for the detection of different phytopathogens (Wallenhammar et al. 2012; Clarkson et al. 2015). Determination of soil pH also gives us an idea of the putative phytopathogens that can be present (Ghosh et al. 2019) as alkalinity and acidity of soil significantly influence diseases like clubroot of crucifers caused by *Plasmodiophora brassicaea* and common scab of potato caused by *Streptomyces scabies*. Similarly, the level of nutrients also gives an idea of potential disease as, for example, higher levels of potassium in soils lessen the chances of occurrence of *Fusarium* spp. (Panth et al. 2020). Soil temperature is a key regulator in disease development; thus, by calculating it, the disease can be predicted (Onwuka and Mang 2018). Genetic background of the cultivar infected



Identification of the Phytopathogen from the Database

Fig. 4.2 Process of identification through molecular methods

also provides us with an initial idea of probable soil-borne phytopathogens that can incite disease in them (Riley et al. 2002). Analysis for the presence and absence of resting and reproductive structures of phytopathogens in the debris of previous crops also serves as a tool for disease diagnosis (Panth et al. 2020). Even the presence or absence of volunteer plants or weeds or alternate/collateral hosts serves as a general way for the detection of soil-borne phytopathogens.

4.3.7 Identification Through Environmental Factors and Prediction Model

For proper detection of disease, it is vital to know the activities that have been performed in and around the infected plant and field. This information contributes to the microenvironment regulation, which is a very important piece in solving the puzzle of disease diagnostics. Each phytopathogen is favored by different sets of environmental conditions, and thus, knowledge about the same completes the disease triangle concept, ultimately providing a way to proceed further for diagnostics of soil-borne diseases. For example, the probability of infection by *Aphanomyces euteiches* in pea growing in moist soil conditions is very high; thus, we can proceed with the idea that the phytopathogen can potentially be *Aphanomyces euteiches* after analyzing the initial symptoms (Clarkson et al. 2015). Prediction models are also a new upcoming tool that is used for the diagnosis of diseases. Environmental factors are fed into machine learning methods and are employed in the detection of soil-borne diseases prior to their onset in prediction models. In this method, various parameters comprising symptoms, morphological parameters, physiological parameters, etc., from the previously diagnosed soil-borne disease are computationally analyzed and stored in the machine database. When a similar set of parameters are observed in plants, the model can successfully predict the disease incidence. Different prediction models have been developed and are already in use for the detection and diagnosis of soybean charcoal root rot (Khalili et al. 2020).

4.4 Future Aspects and Conclusion

For proper management of soil-borne diseases, it is inevitable to properly detect and diagnose the disease-causing phytopathogen. At present, the techniques utilized for the same are faster, sensitive, and reproducible than the conventional ones. However, all the current techniques have their lacunas and are not ready for implementation in field conditions. These techniques rely on heavy and sophisticated instruments, which are unaffordable at the individual level. Moreover, all the current techniques have a much more complicated process than the conventional ones making them

difficult for a person to use without prior scientific know-how. Thus, in the future, the researcher and technology developers have to work together to modify the current techniques to improve their applicability in field conditions and should be simple for the use of ordinary persons without having scientific knowledge. A rapid, mobile, and accurate method or device or tool for diagnosis of soil-borne diseases is essential for monitoring their development and progression to apply management practices timely. This would reduce the chances of heavy crop losses due to those diseases and also reduce the probability of the development of resistance in soil-borne phytopathogens through the judicious application of pesticides.

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