Preface

The diagnosis of infections in plants has changed immeasurably over the years. Early references to disease in barley crops date back to the mid-1500s and probably represent the first true records that we have of symptoms being noted. Early records merely described the physical appearance of plants and were usually associated with catastrophic crop failure. By 1870 fungal and bacterial diseases of plants were diagnosed, but it was not until after 1900 that virus diseases were identified. The science of plant pathology in its true sense came into being when it became possible to treat plants to control pathogens or to use husbandry to avoid pathogen problems. Physical symptoms on plants can be diagnostic, but very often they can be caused by several pathogens and a more scientific approach is required. Diagnostic methods mainly developed for the medical and veterinary sciences have now been applied to diseases of plants and we now have a bewildering assortment of methods at our disposal. Plant diseases still account for heavy losses in many parts of the world where total crop failure due to disease can lead to human misery.

In the western world, much emphasis is now placed on effective disease control by the use of clean seed and appropriate chemical intervention, but both rely on good diagnostics to establish disease status prior to action being taken. The development of quick and cheap methods for disease detection ensures that crop plants remain free of pathogens.

Plant pathology techniques fall into three categories: traditional, serological and nucleic acid, although some span more than one discipline. Traditional methods include the use of indicator plants to produce visual symptoms of disease on susceptible hosts and the use of synthetic media to encourage the growth of microorganisms which can then be identified by colony morphology. These methods can be coupled with more advanced techniques where additional information is required for diagnosis.

Serological methods are all based on the unique ability of animal antibodies to bind to small target areas known as epitopes on the proteins that elicited their synthesis. Antibodies to plant pathogens can be raised in the serum of animals quite easily by immunising them with preparations of microorganisms and then collecting the serum by live donation. Antibodies can be recovered from serum by simple chemistry or chromatography and reagents developed by adding markers such as chromogenic chemicals or fluorescent dyes. Monoclonal antibodies can be made in cell lines, using tissue culture obviating the use of animals for serum production. Such methods have long been established in the medical world and now provide rapid robust tests for pathogens in plant material.

Nucleic acid methods are the newest of the technologies but provide the most exciting possibilities. Methods are all based on the fact that small areas of nucleic acid exist within the genome that are unique to an organism and can be used to identify its presence. Once the sequence of these regions is known, synthetic fragments can
be made which will bind to the specific areas on the pathogen DNA. These areas can then be made to replicate to produce multiple copies that can then be visualised either by electrophoresis or by fluorescent-based methods. The polymerase chain reaction (PCR) is fundamental to the nucleic acid technologies as it provides a way to amplify selected specific regions of DNA so that they can be visualised and thus provide a measurable signal indicating the presence of the target DNA.

This first edition of *Plant Pathology Techniques and Protocols* seeks to provide workers with both basic methods and more advanced techniques for the diagnosis of plant pathogens. Those with limited experience will find easy to use protocols and those with more experience will find methods that they may wish to use as alternatives to those already in place. Methods cover pathogens which cause major problems in crop plants globally. Issues of crop identity and authenticity have become more important in recent years and two chapters have been included which will allow workers to genotype samples from two major food crops. Authors are all active researchers and the methods are those currently being used in their laboratories.

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