Editorial

Biotechnology-based Disease Diagnostics

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One of the benefits of the recent surge in biotechnology-based research in the plant sciences is the development of highly specific assays for disease diagnosis and pathogen detection based on antibodies and nucleic acid probes. Diagnostic tests are being developed for a wide range of plant pathogens, from viroids to nematodes, and are becoming available to plant health professionals and crop managers through public and commercial sectors.

Immunoassays utilizing polyclonal and/or monoclonal antibodies are being used extensively in human medical diagnostics and have demonstrated tremendous utility in providing sensitive and specific analyses. Immunoassays have been improved and simplified over the past decade and now can be performed by persons with minimal training. Test formats based on the double antibody ELISA (enzyme-linked immunosorbent assay), first adapted from clinical diagnostics for plant-pathogenic viruses, are being used for the detection of bacteria, mycoplasmalike organisms, spiroplasmas, and fungi. The extension of this type of assay beyond viruses to other, more complex pathogens is in part a result of the application of hybridoma technology to these microorganisms. Monoclonal antibodies can overcome some of the deficiencies in specificity and affinity that may be problematic with polyclonal antisera. Since monoclonal antibodies are produced by cultured hybridomas, which are theoretically immortal, a constant supply of standardized antibody is available. This is an important advantage over polyclonal antisera, which may vary between animals and between batches from the same animal. Because of their limited and defined specificity, monoclonal antibodies must be rigorously tested against many related and unrelated isolates of the target pathogen. They also tend to be highly assay-specific, and monoclonal antibodies developed using one type of assay may not be appropriate for another format. Although the development of monoclonal antibodies may be expensive and time-consuming for these reasons, the benefits of the technology make it well worth pursuing for diagnostic plant pathology.

Not long ago, the typical ELISA was a fairly slow test, requiring reagent incubations of several hours to overnight. However, improvements in solid-phase supports, purification of antibodies, and enzyme conjugation procedures have contributed to increased sensitivity and reductions in total assay time requirements. Immunoassays complete in 3–4 hours are common in plant pathology, and some can be completed in an hour or less. Simple, sensitive, accurate assays requiring less than 30 minutes to run are a reality in human health care and can be expected to be developed for agricultural applications in the near future. These developments are critical in moving immunoassay technology out of the research laboratory and into the hands of diagnosticians, crop consultants, crop managers, extension personnel, and others responsible for solving crop management problems.

While immunoassays have reached the point of practical application in agriculture, DNA probe technology is just beginning to be established in research laboratories. Probe development is a natural outgrowth of many of the molecular biology programs that have been started in the last 5 years in plant pathology, and we can expect rapid progress in this area. Nucleic acid probes have been developed for members of all the major groups of plant pathogens. Current assay formats rely primarily on the use of radioactive labels, however, and practical application of DNA probes will be limited until alternative markers are found.

Crop systems present unique challenges to the use and interpretation of molecular diagnostic assays. Crop variety, tissue type (stems, roots, leaves, etc.), and the environment affect not only pathogen infection and growth characteristics but also assay performance. Sampling parameters, for individual plants as well as for populations, are crucial and must be worked out for each crop/pathogen system. Detection of pathogens in soil may be complicated by variable soil composition, low concentration of propagules, multiple propagule types, viability, and other factors.

What kinds of applications of immunoassay and nucleic acid probe technology can be reasonably expected during the next few years? Immunoassays are being used routinely today to detect viruses and bacteria in vegetatively propagated plant material for purposes of quarantine, certification, and maintenance of clean stock. They are primarily run in a laboratory environment and require some specialized equipment. They have proved to be efficient, accurate, and economical in supplementing or replacing such labor-intensive bioassays as indicator plant inoculation, growing-on tests, and dilution plating. The use of immunoassays in these types of programs can be expected to increase as immunoreagents and assays for more pathogens become available.

Another setting where immunoassays can be effective is the diagnostic clinic. A number of university plant pathology departments and state laboratories have recently upgraded their diagnostic clinic facilities and programs, and the clinics can now utilize immunoassays on a routine basis. These diagnostic clinics and other well-equipped laboratories will also be most likely to take advantage of nucleic acid probe technology as nonradioactive assays are developed.

Beyond the diagnostic applications I have described, immunoassays are being developed to detect pathogens in presymptomatic plant tissue, soil, and other matrices. Quantitative assays such as ELISA can be used to gather data on pathogen abundance in crop systems, leading to the determination of economic threshold levels and treatment recommendations. The assays can also be used to monitor pathogen levels in a crop over time, allowing the crop manager to treat as needed, not necessarily on a schedule. Expanded use of immunoassays will occur as rapid, field-applicable tests, which require minimal equipment and can be run "plant-side" (more likely in a kitchen, office, or cab of a truck), become more widely available.

Molecular diagnostic assays should be viewed as tools to help manage plant diseases. They are not designed to be used in a vacuum, but rather in conjunction with a knowledge of symptomatology, varietal responses to pathogens, and environmental effects on disease development. As such, they will play a key role in crop management systems, permitting accurate diagnosis of diseases, early detection of pathogens, and more efficient implementation of effective measures of control.