NEW TECHNOLOGY IN PLANT HEALTH TESTING

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The production of healthy, pathogen-free plant materials is a key objective in propagation operations, but one that is not easily achieved. There are many avenues through which pathogens can infect nursery crops, with one of the most insidious being through the plant material itself. There are numerous examples of viral, fungal, and bacterial pathogens being carried on or in apparently healthy plant tissues. Sensitive methods for detecting pathogens in seed or vegetative propagating materials are necessary to insure high standards of plant quality.

While there have been many advances in virus detection over the years, the methods for detecting bacterial and fungal pathogens are much the same today as they were 100 years ago. Detection usually involves some form of culture-indexing, which can be slow, and may require both specialized laboratory facilities and personnel skilled in taxonomic identifications. These constraints have limited the effort and success of screening programs for fungi and bacteria. But recent advances in biotechnology have led to the emergence of relatively simple, highly effective detection methods, which will undoubtedly form the foundation of future screening programs (4, 6). These methods are quickly moving from laboratory phenomena to commercial realities.

IMMUNOASSAY METHODS

Chief among the new technologies is serodiagnostics. This is a familiar approach to pathogen detection, since antibodies have been used to detect viruses in plants for many years. But the application of serodiagnostics to fungi and bacteria has been far more difficult to accomplish (3). This is because fungi and bacteria are far more complex antigenically than viruses. The antigenic complexity of these organisms has made it very difficult to develop antisera with desired levels of specificity and affinity using conventional methods. However, the recent development of monoclonal antibody procedures has largely eliminated these problems (3, 6), while at the same time enabling large-scale commercial production of a highly uniform antibody product. The utilization of highly specific monoclonal antibodies in sensitive ELISA test formats, has enabled the development of detection kits which are simple to use, and which provide rapid, accurate

detection of their target organisms. Some kits now available require no special laboratory facilities and can be performed in a matter of minutes.

While antibodies have been developed recently for a variety of important fungal and bacterial pathogens (e.g. 1, 9), as well as for viruses (e.g. 7) only a few have been commercialized into test kits at this time. I have been evaluating prototype kits produced by Agri-Diagnostics Associates (Cinnaminson, NJ) for the detection of *Phytophthora* spp., *Pythium* spp., and *Rhizoctonia* spp. in nursery plants. These kits are genus-level tests intended to provide broad detection capability. I have found the phytophthora kit to be as effective as culture-plating in detecting several *Phytophthora* species (5). The Pythium and Rhizoctonia test kits appear promising for nursery crops, but will need refinement before they can be used and interpreted effectively in areas outside of propagation.

While monoclonal antibody-based tests offer advantages in speed and simplicity, they also have limitations. For example, some pathogens (e.g. potato spindle tuber viroid) are so simple that they possess no antigenically active components and are impossible to detect by serological methods. Also, antibodies that work well in one assay format, may not work well in another (6), or pathogens may produce different levels of the detected antigen in different hosts or under different environmental conditions. And since the ELISA reaction intensity varies along a continuous scale between "none" to "very strong", one must determine what constitutes a positive test result (8). Background "noise" is common and may vary among plant species and test conditions.

NUCLEIC ACID PROBES

Another detection method that is becoming more common involves techniques of nucleic acid hybridization. Nucleic acid "probes" are developed by extracting and fragmenting nucleic acid (i.e. DNA) from pure cultures of the target organism. A nucleic acid fragment can be inserted into the genome of a bacterium, where it is replicated along with the transformed bacterial genome. Nucleic acid extracts from transformed bacteria are used in hybridization tests to detect the complementary gene in the target pathogen. If the selected gene is unique to the target organism, the probe can be a highly specific detector (2). Furthermore, because the transformed bacteria are typically grown on media amended with ^{32}P to produce radioactive nucleic acid probes, they are much more sensitive detectors than immunological tests. Very little target nucleic acid is required to yield a detectable positive result.

Because of their high degree of specificity, nucleic acid probes can be less subject to background "noise" than serological tests,

but they introduce their own problems. These tests require well-equipped laboratories and radioactive materials can only be used in licensed facilities. There have been efforts to tag nucleic acid probes with enzymes or fluorescent markers to enable wider use, but this invariably degrades test sensitivity. To retain optimum detection capability of the potato spindle tuber viroid in potato seed pieces, Agdia (Mishawaka, IN) has established a centralized testing program, wherein samples are collected in the field, spotted onto membranes and mailed back to the laboratory for radioactive probe analysis. This approach may become more common in the future.

SUMMARY

There are many other diagnostic procedures being developed to simplify and improve the accuracy of pathogen detection, but ELISA tests and nucleic acid probes will clearly dominate the field in the coming years. But even as new diagnostic techniques improve detection capability, they raise questions for which there are few answers. Chief among these is test interpretation. What constitutes a positive test result? Does the presence of a target organism at extremely low levels always indicate a potential disease situation? If reactions are very weak, how can we be sure it is the target organism at low levels, or some other organism which, coincidentally, may have antigens or nucleic acid sequences in common with the target? How can one be sure the tests are detecting a viable pathogen and not residual degradation products of a killed organism? As tests become ever more sensitive, scientists may have to decide if there are acceptable levels of target organisms in plants, much as there are acceptable levels of certain chemicals in human food or water supplies. These are questions which have not been answered because we have never previously had tests sensitive enough to make them an issue, and researchers have not had wide access to the tests needed to undertake the necessary experiments.

It also must be kept in mind that these tests are essentially "recognition" reactions. A mutation in a single antigen or nucleic acid sequence in the pathogen could render a test ineffective long before anyone recognizes the failure. But even with their potential ambiguity and fallibility, these tests are far superior to current culture plate methods. This is an area of increasing research activity, and one which will significantly affect those involved in plant health testing over the next decade

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