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An Over View of Conventional and Molecular Detection Methods of Seed-Borne Bacterial Pathogens

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Review Article

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ABSTRACT

The detection of pathogenic seed borne bacterial pathogens is an important aspect of disease management. Presence of diseased seeds in seed-lots cannot be reliably detected by visual examination. Radiographic assays of seeds provide an efficient, non-destructive method to determine internal seed damage. However, with advances in molecular techniques, emphasis in bacterial identification and taxonomy has changed from conventional approach to a more molecular approach. DNA analysis techniques such as the polymerase chain reaction (PCR) and Random Amplified Polymorphic (RAP) analysis are the most commonly used tools. Nucleic acid extraction is still necessary in many cases and in practice inhibition problems are decreasing the theoretically sensitivity of molecular detection. Overall, molecular techniques based on PCR amplification and very especially on real-time PCR are leading to high throughput, more rapidly and more accurate detection methods for the most severe seed borne bacterial pathogens

INTRODUCTION

Seed is the most important input for crop production. Many plant pathogens are seed borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops. Seeds are the most important source of primary inoculum for many bacterial disease outbreaks [1,2]. Determining the presence of seed borne pathogens allow managers to apply the appropriate controls or modify management practices to avoid the problem in the future [3]. As a result, pathogen free healthy seed is urgently needed for desired plant populations and good harvest. Seed health testing is thus routinely carried out in most countries for domestic seed certification, quality assessment and plant quarantine [4]. Seed health testing is an integral for all seed companies in disease risk management and improves germination potential of seed which finally leads to increase of the crop production [5].

Bacteria have been recognized as a distinct group of plant pathogens capable of inducing significant losses in various crops cultivated in different countries all over the world. Various methods based on their morphological, biochemical/ physiological, immunological and nucleic acid characteristics have been applied for their detection, differentiation and identification with different levels of sensitivity, specificity and reliability [6].

Bacterial plant pathogens are known to be carried on or in the true seeds and vegetative propagated plant materials such as tubers, corms, cuttings and setts that form the primary sources of infection for the crops grown in the next season [7]. It can survive in or on the seed longer the seed itself. Infection of approximately 1 in 10,000 seeds is capable of causing an outbreak of bacterial disease [8]. Therefore, it is necessary to detect the bacterial pathogens in these planting materials to prevent the introduction of new bacterial pathogen(s) into the new areas or to restrict the spread of the existing bacterial pathogens in a geographical location [9]. Timely detection and appropriate identification of the causal agents associated with disease of seeds are consider to be the most important issue in formulating sustainable management of plant bacterial diseases.

LITERATURE REVIEW

From Conventional to Molecular

Infection of seeds by bacterial pathogens may not be recognized by visual examination in most cases. Internal seed contents can be examined by cutting the seed open and looking for symptoms of disease [10]. Seed borne pathogens can also be present on

seeds without obvious disease symptoms [11]. As a result many detection methods have been developed over the years for various seed-borne bacterial pathogens. However, the detection of seed borne bacterial pathogens still largely depends on too classical activities like morphological identification, microscopical observation, biochemical characterization and cultural assets [12]. The nucleic acid based detection method has emerged now to overcome and supplement these restricted accesses.

Biological Assays

This procedure is highly selective as it relies on the specificity of the host pathogen interaction. Its sensitivity is less assured, as inoculation thresholds may vary depending on the plant cultivar being tested, fluctuations in environmental conditions, fertility, and other factors. Additionally, the ability of plant inspectors to reliably detect low incidences of disease is a critical factor, as it is often necessary to visually recognize single lesions in thousands of plants. Nonetheless, grow-outs are widely used and accepted as definitive in determining the infection status of a seed lot. Because grow-outs rely on symptom expression, a positive result usually is irrefutable evidence that the bacterium was present, viable, and pathogenic [13]. On the other hand, standard protocols for detection of seed borne bacterial pathogens based on isolation and further identification are time consuming and not always sensitive and specific enough. Consequently, they are obviously not suited for routine analysis of a large number of samples [12]. They have low reproducibility of identification phenotypic traits and regular lack of phylogenetic significance [14].

Nucleic Acid-Based Techniques

The selection of detection methods depends upon the purpose of the test i.e., whether the seeds are to be tested for seed certification, seed treatment, quarantine etc. If for quarantine purposes, then highly sensitive methods are preferred. In the case of conventional seed borne disease detection method, seed assays have been developed based on different technologies including visual examination; selective media; seedling grow-out tests and serological techniques. At the same time, it becomes necessary to employ special techniques for the detection of bacterial infection in seeds like nucleic acid-based techniques [15].

The most commonly applicable nucleic acid based techniques now days are Nucleic Acid Hybridization Technique, Polymerase Chain Reaction-Based Assays, Real-Time Polymerase Chain Reaction, and Restriction Fragment Length Polymorphism Analysis. Development of diagnostic DNA probes specific for the pathogen(s) concerned has helped in detecting, differentiating, and quantifying the population of bacteria inside the seed very rapidly, especially the pathogens known to be transmitted through seeds and other planting materials. The wide acceptability of nucleic acid based techniques is due to them being more sensitive, perfect, detailed and much faster than the conventional methods. Methods like real-time PCR, multiplex PCR, and Bio PCR are among the detection options that provides rapid data analysis with specificity. Real-time PCR has been applied to successfully identify and quantify many phytopathogens with improved sensitivity compared to culture plate testing and conventional PCR [16]. Therefore, there is a need to develop and apply real-time PCR tests in routine seed health testing to increase efficiency, specificity, and sensitivity [12, 6, 16].

DISCUSSION AND CONCLUSION

Generally, every pathogen detection methods starting from conventional up-to the most sophisticated real-time PCR have their own advantages and drawbacks but selecting of the most appropriate one based on its multi-dimensional importance is crucial at this time and integrated approaches including 16S rDNA sequencing, Biolog-phenotyping, fatty acid methyl esterase profiling and pathogenicity test are to be recommended. Development of RNA microbes, which enable gene expression measurements of thousands of genes from seed borne bacterial pathogens, will provide data for selecting new markers for detection. The accuracy of new detection protocols based on molecular will lie behind the availability of seeds free of a wide range of bacterial pathogens in the near future.

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