DEVELOPMENT OF MOLECULAR TECHNIQUES TO IDENTIFY SEEDBORNE PATHOGENS

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Abstract

Numerous, important seedborne organisms affect the major row crops produced throughout the cotton producing areas. In addition to cotton, the major rotational crops that include barley, corn, oats, peanut, rice, rye, sesame, sorghum, soybean, and wheat, are all affected by seedborne bacterial, fungal, and viral organisms. The most commonly associated seedborne organisms are fungal. In fact, approximately 73% of the commonly observed seedborne organisms in 62 important plants are caused by fungi. Even though seedborne organisms can be quite common, detecting seedborne organisms, regardless of the particular plant affected, can be a difficult procedure. Fungal organisms that produce a specific outward symptom or sign on the seed itself can either be observed with the naked eye or the help of a microscope. Numerous fungi can be easily identified by using standard microbiological growth media in the laboratory. In addition to the standard observational methods that can be used for the purposes of confirming the presence of seedborne organisms, serological techniques, namely enzyme linked immunosorbent assay methods (ELISA) are available for bacterial, fungal and virus diseases of numerous host plants. However, for some organisms the ELISA-based methods are not specific enough since species-specific antibodies are not available. More refined, and ultimately specific detection methods relaying on molecular procedures are necessary for some organisms; however, the tests have yet to be developed.

Since 2011, the Mid-southern United States has been affected by widespread bacterial blight of cotton. Bacterial blight, caused by *Xanthomonas citri* pv. *malvacearum* (*Xcm*), produces characteristic symptoms on all plant parts in the field. However, the organism can become seedborne and spread the disease to areas where cotton has not previously been grown or is not a common component of a rotation. As a result of two years of bacterial blight in several midsouthern states, Cotton Incorporated funded a project to develop a molecular screening technique to determine the bacterial blight organism in affected seed. Prior to the development of a quantitative method to positively identify the bacterial blight organism in infested seed, the in vitro methods required to screen for the organism were laborious and no single published method exists. To develop the pathovar-specific quantitative PCR (qPCR) detection method, we sequenced the local strain MSCT1, which was isolated from the Mississippi Delta. A draft of the genome sequence of the United States strain of *Xcm*, MSCT1, was completed, which yielded a 5 Mb circular scaffold. A total number of 4,247 protein coding genes were identified. Two plasmids were predicted from the sequence analysis. Based on a genome sequence comparison, a few pathovar-specific DNA regions were identified. One pair of pathovar-specific primers and a Taqman probe were designed from the DNA regions. TaqMan-based, real time PCR were performed to test the sensitivity and efficiency of the probe. When genomic DNA of MSCT1 was used for amplification, the efficiency for the standard curve was 104%. The assay exhibited a linear response (R^2=0.998). The detection limit of the reaction was one cloned copy of the target gene of *Xcm*. Using the DNA extracted from artificially infested cotton leaf and seed samples, the qPCR analysis successfully detected the causal pathogen and no PCR amplicon was observed from negative controls. The detection limit is 102 copies from 1 g fresh leave samples; and 37 copies from 1 g of seed. These results suggest the qPCR primers and probe can be used to detect the bacterial blight pathogen from cotton plant material including for seed quality control.