

2001 EXECUTIVE SUMMARY REPORT

PROJECT TITLE: CONTROL OF BACTERIAL DISEASES OF TOMATO SEED AND PLUG PLANTS THROUGH THE DEVELOPMENT OF IMPROVED DIAGNOSTIC TOOLS

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PROJECT OBJECTIVES:

- 1) Develop a PCR-based assay, employing two or more primer sets for the highly heterogeneous bacterial spot pathogen, that will detect one spot bacterium in a large tomato or pepper seed sample (20,000 to 15,000 seeds) regardless of whether or not the seed has been coated, fungicide-treated or pelletized;
- 2) Develop an equally-effective PCR-based assay for bacterial canker or speck-infested seed.

(Once detected, infested material can then be treated with a bactericide or destroyed, thus limiting the spread of disease to non-contaminated plants or seed and reducing crop damage)

METHODOLOGY:

Seed processing and DNA extraction. Prior to germination, pelleted or coated seeds were washed in sterile water to remove as much of the coating as possible; raw seeds were not washed. Seeds then were germinated by incubating them in a moist chamber at 25 C for 4 days. Germinated seeds were weighed and then washed in sufficient buffer to cover the sprouted seed. The buffered wash water was separated from the seed and infested with various concentrations of the bacterial spot, bacterial speck or bacterial canker pathogens. Bacterial DNA was then extracted and purified from the wash water by a protocol we developed previously. No bacteria were added to the control samples.

PCR (polymerase chain reaction) assay for detecting bacterial spot, speck and canker. The presence of these pathogens on the various batches of seed was determined by PCR using a GeneAmp® PCR System 9700 thermal cycler and a) the spot-specific AAFC-BSX primers developed by our laboratory, b) the spot primers developed by our collaborator J. Jones (Univ. of Florida), c) the *fimA* primers (Van Doorn et al., 2001), d) the speck-specific AAFC-COR1/2 primers (our laboratory) and e) the canker-specific primers CMM3/4 (Rademaker and Jansen, 1994).

RESULTS:

Development of a PCR-based seed assay which is highly sensitive.

We have discovered that a PCR-based seed assay for bacterial disease is much more sensitive if the seeds are first germinated, allowing the bacteria to emerge from their dormant state. Germination increases the bacterial spot population approximately 100,000 times. Although we cannot PCR-detect the pathogen on dry, non-germinated seed when the bacterial numbers drop below approximately 3000/g of seed, we can easily detect one viable bacterium in 20,000 raw seeds if the seeds are first germinated. Previously, the sensitivity of our assay was one viable bacterium in 10,000 raw seeds; we were able to increase the sensitivity by refining our DNA extraction protocol.

Our current detection limit for pelleted seed is approximately ten viable bacteria in 20,000 seeds. The PCR-based assay worked effectively with chlorine/Thiram-treated, acid/Thiram-treated and chlorine-hot water-tri-sodium phosphate-treated seed; it also functions well with pepper seed samples. To date, we have tested the assay with 3 tomato varieties of raw seed, 6 tomato varieties of pelleted seed and 3 pepper varieties of coated seed. One of the pepper seed samples, originating from a 1998 seed lot, was naturally infested with bacterial spot and tested positive; the controls (not artificially infested) for the remaining samples were negative.

Identification of a second PCR primer set for bacterial spot.

Because new forms of the bacterial spot pathogen continue to appear and are, in fact, inevitable, it is essential that we have a second set of diagnostic primers for bacterial spot to ensure that we are detecting all forms of this important tomato pathogen. In addition to the AAFC BSX primers for bacterial spot, we tested the RST65/69 primers (Univ. of Florida, unpublished data) and the *fimA* primers (Bulb Research Centre, The Netherlands). As DNA templates, we used a selection of strains from our bacterial spot culture collection, including strains we have isolated from diseased Ontario tomato and pepper plants over the past eight years (86 strains). Although the AAFC-BSX primers amplify the majority of bacterial spot strains, they do not recognize T3 strains. Fortunately, the RST65/69 do amplify T3 strains, as well as all of the other strains we assayed. The *fimA* primers appear to recognize only group B or Race T2 strains. Both the AAFC-BSX and the RST65/69 primers readily identified the *X. gardneri* strains responsible for the bacterial spot disease seen in 2000.

Development of an equally-effective PCR-based assay for bacterial speck and canker.

Each PCR primer set has a unique set of characteristics (size, melting temperature, sequence composition, and physical traits) and thus substances on germinated seed that have no effect on the bacterial spot primers may seriously hamper amplification with the bacterial speck PCR primers AAFC-COR1/2 or the bacterial canker PCR primers CMM 3/4 (first used by Rademaker and Janse, 1994, Can. J. Microbiol. 40:1007-1018). We infested seed preparations (20,000 seeds/sample) with the bacterial speck and bacterial canker pathogens and tested AAFC-COR and CMM3/4 with DNA extracts from these preparations. We found that the large-seed-sample protocol that we had developed for the bacterial spot pathogen works equally well with these other pathogens.

SUMMARY AND CONCLUSIONS

1. Our PCR-based protocol allows us to detect 1 viable bacterial spot cell in 20,000 raw tomato seeds. For pelleted seed, which are covered in an amalgam of fillers (e.g., clay) and cementing additives, the detection level is slightly less at 10 viable bacteria in a 20,000-seed sample.
2. Various seed treatment chemicals, such as Thiram, chlorine, and hydrochloric acid, do not affect the PCR assay.
3. The PCR assay also works effectively with large samples (15,000) of coated pepper seed.
4. The Florida PCR primers that we tested, RST 65/69, recognized every bacterial spot strain that we tested, including race T3 strains, whose DNA is not amplified by the AAFC-BSX primers. The *fimA* primers, although developed for *X. vesicatoria*, cannot be used for detection or disease diagnosis.
5. The large-seed-sample PCR protocol that we have developed for the bacterial spot pathogen works equally well with the bacterial speck and bacterial canker pathogens.