WEILAND, JOHN J.* Sugarbeet and Potato Research, USDA-ARS-Northern Crop Science Laboratory, Fargo, N.D. 58105. **Discrimination of sugarbeet fungal pathogens using amplified DNA from regions of the actin and rRNA genes.**

## ABSTRACT

Determination of the causal agents of seedling damping-off and adult root rot can be confounded by misclassification of the disease-causing organism and by the presence of co-colonizing saprophytes. The actin and nuclear ribosomal RNA (rRNA) genes were used as targets in the development of polymerase chain reaction (PCR) protocols that permitted discrimination of common sugarbeet fungal pathogens without the need for phytopathological expertise. Using DNA primers (5FWDACT and MIDREVACT) directed to conserved regions in the actin gene, amplified DNA was generated from genomic DNA prepared from *Aphanomyces cochlioides*, *Pythium ultimum*, *Rhizoctonia solani*, *Fusarium oxysporium*, *Phoma betae*, and *Cercospora beticola* that was of a size consistent with the amplification of actin gene sequences. Use of primers ITS1 and ITS4 in the amplification of the internal transcribed spacer (ITS) region of the nuclear rRNA gene of these fungi also yielded products consistent with the amplification of this gene region. Size polymorphisms in the DNA amplified with the actin and rRNA primer pairs observed between pathogens in different genera also were consistent with the known sequence diversity that exists within these two genes. Where amplified product DNA size was indistinguishable between any two members of differing fungal genera, restriction fragment length polymorphisms (RFLPs) observed after restriction endonuclease digestion of the amplified DNA permitted discrimination of the pathogens. Use of the assay in the detection of *A. cochlioides* in infected sugarbeet seedlings is presented.

### Objectives

The objectives of the project were to determine whether sufficient sequence diversity exists within the actin and rRNA genes to use for DNA-based pathogen discrimination. Additionally, protocols were tested for the detection of *A. cochlioides* in diseased sugarbeet seedlings using the polymerase chain reaction (PCR).

### Procedures

The actin genes of several filimentous fungi were compared by computer using sequences stored in public sequence databases. Highly conserved regions of the gene were identified by this alignment and DNA primers were synthesized based on these conserved regions. The primers were used with standard PCR conditions in the amplification of actin sequences from the genomic DNA of the sugarbeet fungal pathogens *Aphanomyces cochlioides*, *Pythium ultimum*, *Rhizoctonia solani*, *Fusarium oxysporium*, *Phoma betae*, and *Cercospora beticola*. The rRNA gene ITS region also was targeted for discrimination of these fungi using PCR. Both the rRNA and actin genes are highly conserved in eukaryotes.

Products of the amplification reactions were fractionated by agarose gel electrophoresis. For both actin and rRNA gene-based primers, amplified products were of a size consistent with the bona fide amplification of the two genes, respectively. In some cases, the products amplified from the

genomic DNA of one pathogen was of a size similar to that for another pathogen. In these cases, product DNAs were digested with 4-base cutter restriction endonucleases in order to sample sequence diversity within the product. Digested DNA was fractionated on 6% native polyacrylamide gels generating DNA fingerprints enabling these pathogens to be distingushed.

The facile amplification of actin gene sequences from *A. cochlioides* DNA combined with the lack of amplification of sugarbeet genomic DNA under the same conditions suggested the possibility that *A. cochlioides* might be detected in diseased tissue by PCR. Conditions for the extraction of DNA from diseased tissue required the inclusion of reagents to remove inhibitors of the PCR reaction. By using stringent anneal conditions, a PCR protocol was devised that permitted the amplification of *A. cochlioides* sequences without detectable amplification of sugarbeet genome sequences.

**Conclusions**

Sufficient diversity exists within the actin and rRNA genes to consider their use as substrates for PCR-based diagnostics. Where the amplified products of one of the genes (e.g. actin) were of a similar size between two organisms, either digestion with restriction endonucleases or comparison of the amplified products of the other gene (e.g. rRNA ITS) aided in pathogen discrimination. The presence of these pathogens within soil or plant debris in quantities too low to be detected by traditional means might be detected using future protocol employing PCR.