

Development of PCR Assay Using Species-Specific Primers of *Phytophthora sojae* Based on the DNA Sequence of Its Transposable Element

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ABSTRACT

Phytophthora stem and root rot caused by *Phytophthora sojae* is one of the most important diseases of soybean (*Glycine max*) in the world. Polymerase chain reaction (PCR) was used for the specific detection of *P. sojae* in soybean plants. A primer set, PS12 and PS6R, was designed from nucleotide sequences of a Gypsy-like retroelement of *P. sojae* that was repetitive in its genome. PCR amplification using the primer set PS12 and PS6R produced a 282 bp PCR product exclusively to 25 *P. sojae* isolates representing 13 races, but not for DNA from 17 other *Phytophthora* species, several other pathogens of soybean, and soybean. The sensitivity limit of the primer set was approximately 100 fg, and these primers detected the pathogen at a concentration of one zoospore and oospore per ml of water. In addition, the PCR primer set detected *P. sojae* from diseased soybean stems and roots obtained from greenhouse inoculated plants and from infected fields plants. This PCR detection method provided a rapid and accurate diagnostic tool for detection of *P. sojae*.

INTRODUCTION

- Phytophthora sojae* causes root and stem rot of soybean.
- One of the most important soilborne pathogens of soybean.
- Difficult to control as oospores overwinter in soil for many years.
- Need rapid and accurate identification method for diagnosis and management.
- Development of species-specific primer for *P. sojae* from Gypsy-like retroelement.

The purpose of this study was to develop a rapid and accurate PCR method using a species-specific primer for identification and detection of *P. sojae* from plants and soil.

MATERIALS AND METHODS

- DATA search – JGI genome web site
- Primer design
- DNA structure – ORF analysis
- DNA isolation – CTAB and NaOH method
- Isolation of single spore – zoospores, oospores
- PCR – conventional PCR
- Inoculation on soybean
- Infested soil with pathogen
- Multiplex PCR with other pathogen primer sets

RESULTS

Species-specific primers from retroelement region in *P. sojae* genome

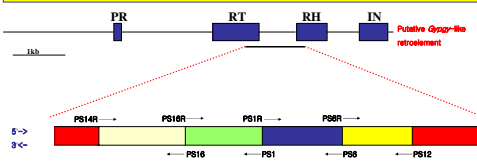


Fig. 1. Primer sets designed from retroelement in *Phytophthora sojae* genome.

Specificity of selected primers (PS) from retroelement regions of *P. sojae*

