Development of PCR Assav Using Species-Specific Primers for 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. soige 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

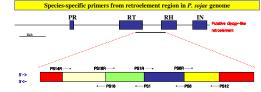


Fig. 1. Primer sets designed from retroelement in Phytophthora soiae genome.

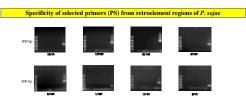


Fig. 2. Primer sets designed from retroelement of Phytophthora sojae genome. Test pathogens; CT, PG, RS, PM, PPAR, PFRA, PCAC and PS (see Tables 1 and 2 for names).

PCR amplification of DNA samples using ITS1/ITS4 and PS12/PS6R

Table 1. Phytophthora species used in this stud

| | | PCR | PCR |
|--------------------------|----------------|-----|-----|
| P. boehameriae (PBOE) | Tree of heaven | - | + |
| P. cactorum (PCAC) | Ginseng | - | + |
| P. cambivora (PCAM) | Apple | - | + |
| P. capsici (PCAP) | Pepper | - | + |
| P. cinnamowi (PCIN) | Japanese larch | - | + |
| P. citricola (PCTI) | Cherry | - | + |
| P. citrophthora (PCTO) | Cocoa | - | + |
| P. colocusiae (PCOL) | Cocoa | - | + |
| P. cryptogea (PCRY) | Gerbera | - | + |
| P. drechsleri (PDRE) | Pigeon pea | - | + |
| P. erythroseptica (PERY) | Potato | - | + |
| P. fragariae (PFRA) | Strawberry | - | + |
| P. infestans (PINF) | Potato | - | + |
| P. mirabilis (PMIR) | Four o'clock | - | + |
| P. parasitica (PPAR) | Sesame | - | + |
| P. phascoli (PPHA) | lima bean | - | + |
| P. syringae (PSYR) | Apple | - | + |
| P. sojae (PS) | Soybean | + | + |



Table 2. Plant pathogens used in this study

| Isolate | Host plant | PS12/PS6R PCR | PCR |
|--|------------|------------------|-----|
| Cercospora sojina (CS) | Soybean | - | + |
| Colletotrichum truncatum (CT) | Soybean | - | + |
| Diaporthe phascolorum (DP) | Soybean | - | + |
| Erysiphe polygoni (ER) | Soybean | - | + |
| Fusarium solani f. sp. glycines (FU) | Soybean | - | + |
| Macrophomina phaseolina (MP) | Soybean | - | + |
| Penicillium sp. (PN) | Soybean | - | + |
| Phialophora gregata (PG) | Soybean | - | + |
| Phomopsis longgicola (PM) | Soybean | - | + |
| Pythium sp. (PY) | Soybean | - | + |
| Rhizoctonia solani (RS) | Soybean | - | + |
| Sclerotinia sclerotinia (SS) | Soybean | | + |
| Phakopsora phachiryzi (PP) | Soybean | - | N |
| Colletotrichum gleosporioedes (CTG) | Pepper | - | + |
| Stenphylium vesicarium (STE) | Pepper | - | + |
| Fusarium oxysporum f. sp. lycopersici (FLY) | Tomato | - | + |
| F. oxysporum f. sp. radici-lycopersici (FRA) | Tomato | | + |



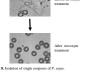
R33, R41, R44, R47, KPSO, negative

PCR test of primer sets PS12/PS6R designed for P. sojae isolates

Table. 3 Phytophthora sojae isolates representing races

| Isolate PS61 | Race | ITS1/ITS4 test | S12/S6R test | |
|-----------------|------|-------------------|-----------------|--|
| | | | | |
| | 1 | + | + | |
| PS71 | 1 | + | + | |
| PS72 | 3 | + | + | |
| PS73 | 3 | + | + | |
| PS39 | 3 | + | + | |
| PS42 | 4 | + | + | |
| PS62 | 4 | + | + | |
| PS12 | 7 | + | + | |
| PS75 | 7 | + | + | |
| PS76 | 7 | + | + | |
| PS46 | 17 | + | + | |
| PS77 | 25 | + | + | |
| PS78 | 26 | + | + | |
| PS28 | 28 | + | + | |
| PS59 | 28 | + | + | |
| PS79 | 28 | + | + | |
| PS80 | 30 | + | + | |
| PS60 | 33 | + | + | |
| PS47 | 33 | + | + | |
| PS50 | 41 | + | + | |
| PS56 | 43 | + | + | |
| PS36 | 44 | + | + | |
| PS58 | 47 | | | |
| KPSO | VND | | | |

PCR sensitivity of primer set PS12/PS6R



Application of species-specific primers of P. sojae















cies: PCAP PCAC, PCIN, PCRY, PCOL

SUMMARY

- 1. A specific and sensitive a primer set (PS12/PS6R) was developed to identify and detect P. sojae in plant tissue.
- 2. Species-specific primer sets were designed from the genomic DNA Gypsy-like transposable element region of P. sojae. DNA sequences of primers (>20) in the P. sojae genome were not similar to DNA regions of the P. capsici and P. ramorum genomes.
- 3. The specificity of the primer set PS12/PS6R was a 282 bp product for all 25 P. sojae isolates representing 13 races but not for 17 Phytophthora species, 16 phytopathogens and sovbean.
- 4. PCR sensitivity of primers PS12/PS6R was 100 fg, and detected a single zoospore and oospore.
- 5. The primer set detected P. sojae from diseased soybean plants (inoculated or field-infected) and could be used in multiplex PCR with species-specific primers for other soybean pathogens.
- 6. This PCR assay using species-specific primers was used to identify and detect P. sojae, and could be helpful for diagnosis and developing control strategies.