DNA extraction techniques and sensitive PCR protocols: tools for molecular plant disease diagnostics

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Introduction. As an international shipping hub, South Florida is continuously threatened by introduced plant pathogens. Many of these introduced pathogens have lead to widespread epidemics that potentially threaten an entire crop. Recent notorious examples include citrus canker, citrus greening, Texas phoenix palm decline, soybean rust, and laurel wilt. In order to track disease spread and successfully manage outbreaks, rapid and sensitive pathogen detection methods are a necessary part of disease diagnosis.

Our current research seeks to improve existing *in planta* molecular detection methods by comparing successful detection of pathogen DNA using several common DNA extraction methods coupled with standard or High-Fidelity (Hi-Fi) PCR protocols. Hi-Fi PCR utilizes a second proofreading DNA polymerase with *Taq* polymerase to increase product sizes as well as the sensitivity of the PCR reaction. It has been used to successfully detect *Candidatus* Liberibacter asiaticus in the psyllid vector *Diaphorina citri* (Manjunath et al., 2001) and several pathogens from orchids (Cating, 2010).

Figure 1. Hi-Fi and Standard PCR were used to detect *P. nicotianae* in symptomatic *Spathiphyllum* plants.

Materials and Methods. Leaf, petiole, and root tissue from four symptomatic *Spathipyllum* plants inoculated with *Phytophthora nicotianae* (Figure 1)were harvested for DNA extraction using four different extraction methods:

1. DNeasy Plant Mini Kit (Qiagen)
2. Extract-N-Amp Plant Kit (Sigma-Aldrich)
3. CTAB buffer (20mM CTAB, 0.14M D-sorbitol, 0.8M NaCl, 20mM EDTA, 0.1 M Tris-HCl pH 8.0, 1% SDS, 1% PVP) with chloroform extraction and ethanol precipitation
4. Shorty buffer (1M Tris-HCl ph 9.0, 2M LiCl, 0.5M EDTA pH 8.0, 10% SDS) with isopropanol precipitation.

Both Standard and Hi-Fi PCR reactions were used for each DNA sample to detect *Phytophthora nicotianae* using species specific primers DC3/DC8 (Boersma et al 2000) in 50 µl reactions with the following parameters:

1. **Standard PCR:**

   Reaction contents. 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1 % Triton X-100, 0.2 mM dNTPs, 15 pmol primers, 1 unit *Taq* DNA polymerase, 2µl DNA template.

   Reaction conditions. i) preheating and denaturing at 95°C for 2 min; ii) 30 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min; iii) final elongation at 72°C for 10 min.

2. **Hi-Fi PCR:**

   Reaction contents. 50 mM Tris pH 9.0, 16 mM $(NH_4)_2SO_4$, 1.75 mM $MgCl_2$, 350 uM dNTPs, 800 pmol primers, 1 unit Accuzyme (Bioline), 5 units *Taq* DNA polymerase, 2µl DNA template.

   Reaction conditions. i) preheating and denaturing at 94°C for 2 min; ii) 10 cycles of denaturing at 94°C for 10 s, annealing at 55°C for 30 s, and extension at 68°C for 1 min; iii) 25 cycles of denaturing 94°C for 10 s, annealing at 55°C for 30 s, and extension at 68° for 1 min plus an additional 20 s during each consecutive cycle.

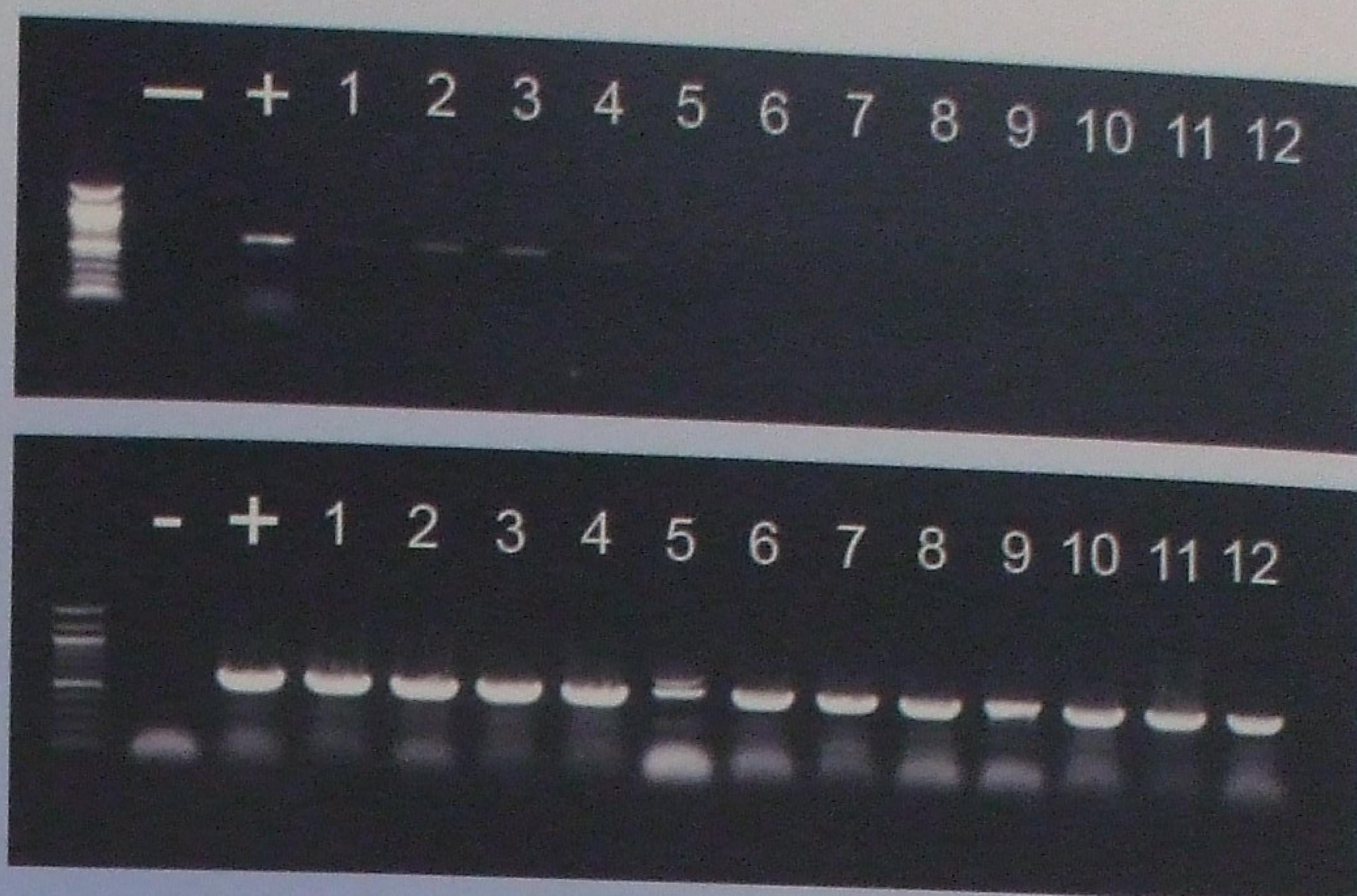

Figure 2. Detection of *P. nicotianae* DNA using Standard (top) and Hi-Fi (bottom) PCR from symptomatic leaves (lanes 1-4), petioles (lanes 5-8), and roots (lanes 9-12). Results shown are using DNA extracted with Qiagen DNeasy Plant Mini Kit. Positive and negative controls precede experimental reactions.

Results and Discussion. Hi-Fi PCR positively detected *P. nicotianae* DNA in all samples of all tissues using DNeasy Plant Kit and Extract-N-Amp Plant Kit, while Standard PCR only detected the pathogen in the leaf samples using the Dneasy Kit with a faint positive (Figure 2). Extraction with CTAB resulted in no positives using Standard PCR, and positives for all but two samples using Hi-Fi PCR. Extraction with Shorty buffer did not produce any positive detection of the pathogen from any sample using Standard PCR, and only one leaf sample was positive using Hi-Fi PCR.

These results indicate that Hi-Fi PCR is more sensitive in detecting pathogen DNA from symptomatic host tissue samples. Our study also indicates that the DNA extraction method, Extract-N-Amp, produces similar results as the DNeasy Kit, although DNA purity may be lower. The Extract-N-Amp protocol requires minimal tissue processing and takes less time to complete.

One clear advantage of Hi-Fi PCR is that it does not require special equipment, making it less costly than other highly sensitive molecular detection methods (i.e. real-time PCR). When combined with species specific primers, Hi-Fi PCR enables detection of specific pathogens directly from plant tissue in a matter of hours (Figure 3), which is especially useful for time sensitive plant diseases.

Future studies will investigate the potential for using Hi-Fi PCR as a more sensitive detection method for other economically important pathogens in south Florida, including citrus greening, lethal yellowing of palms, and laurel wilt of avocado.

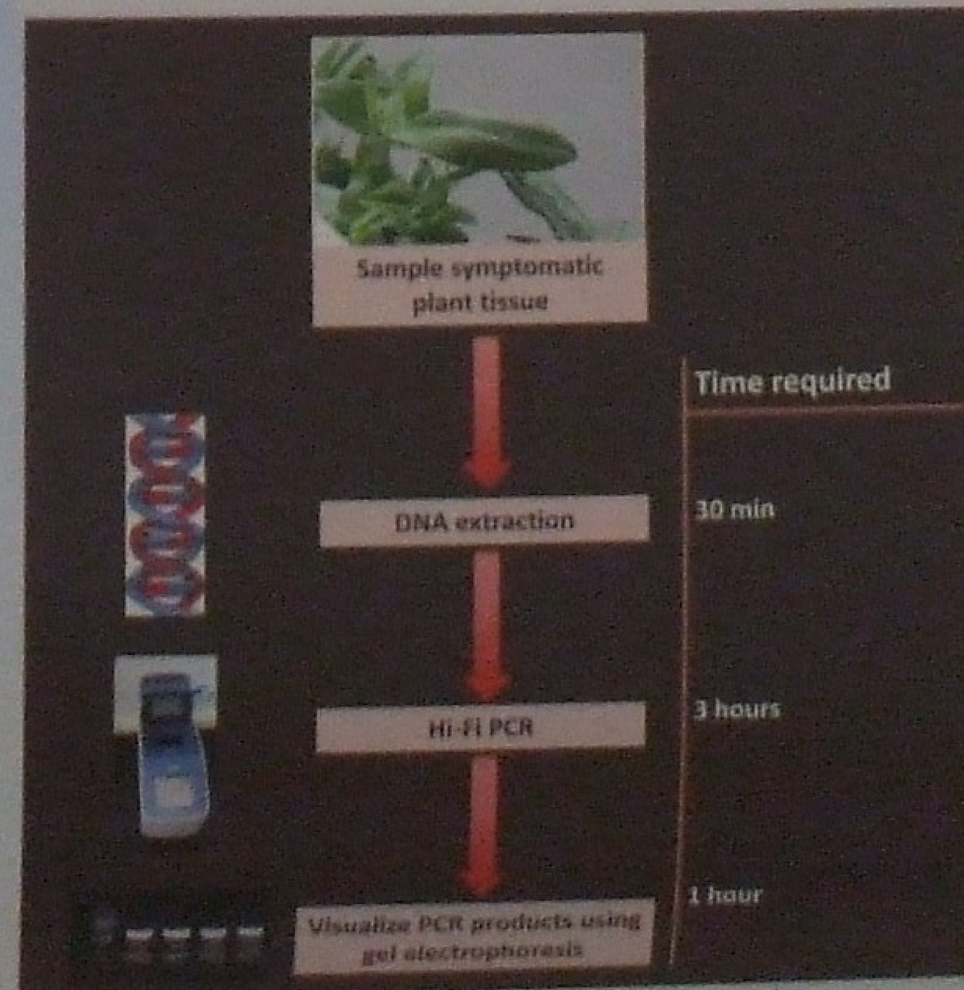

Figure 3. Flowchart of steps involved in molecular detection of pathogen directly from host tissue and the time required for each step.

References

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