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

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The detection and identification of economically important plant pathogens is of paramount importance in Florida and the Caribbean region. Invasive pathogens are particularly grave threats, as exemplified by the recent establishment of the citrus canker, soybean rust, citrus greening, texas phoenix palm decline, and laurel wilt diseases in Florida. Accurate and rapid identification of such threats is fundamental to protecting agriculture. To meet this challenge plant disease diagnostic labs require tools that are effective in detecting plant pathogens. Our recent studies, have included testing DNA extraction techniques including 1) Extract-N-Amp Plant Kit (Sigma-Aldrich), 2) DNeasy Plant Mini Kit (Qiagen), 3) CTAB buffer, and 4) lithium chloride Shorty buffer in conjunction with conventional and High-Fidelity (Hi-Fi) PCR protocols. Results from our research will indicate which DNA extraction methods yield the highest DNA amounts, the purity of extracted DNA, and how this influences PCR sensitivity. Further, we will identify a PCR protocol that is highly sensitive in detecting select plant pathogens in planta, and discuss its potential for routine use in the plant disease diagnostic clinic.

Key Words: molecular diagnostic techniques, invasive species, PCR, High-Fidelity PCR

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