COLORIMETRIC LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR THE DETECTION OF *BRETZIELLA FAGACEARUM* (CAUSAL AGENT OF OAK WILT DISEASE) IN TREE SAMPLES

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PURPOSE AND SCOPE

The purpose of this study was to develop a rapid and efficient diagnostic kit for detecting oak wilt, a devastating disease caused by the fungus *Bretziella fagacearum* (Juzwik et al. 2008). This research aimed to create a colorimetric loop-mediated isothermal amplification (LAMP) (Li et al. 2017) kit that can provide quick visual confirmation of the pathogen within 30 minutes, coupled with a simplified DNA extraction protocol. The scope includes testing the kit's specificity and sensitivity with real oak tree samples and ensuring its effectiveness for large-scale testing during the oak wilt season. The study also focuses on the practical application of the kit in field and lab settings by freeze-drying the reaction mixture and primers for easier transport and minimal error.

METHODS AND APPROACH

The study employed the LAMP technique, known for its rapid and efficient DNA amplification capabilities under isothermal conditions. The LAMP method involves the use of a set of six specially designed primers that recognize six distinct regions of the target DNA, ensuring high specificity and sensitivity. For the detection of *Bretziella fagacearum*, specific primers targeting the pathogen's unique DNA sequences were designed and tested.

A colorimetric LAMP assay was developed, where a color change from pink to yellow indicates the presence of the pathogen. The LAMP reaction mixture and primers were freeze-dried on reaction tubes to facilitate easy transport and on-site testing. A simplistic DNA extraction protocol was also developed, allowing DNA to be extracted from wood samples within 20 minutes using minimal equipment.

The assay was tested on 39 oak tree samples, showing a specificity of over 90 percent and a sensitivity of 100 percent. The entire detection process, including

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DNA extraction and amplification, can be completed in under 45 minutes, making it highly suitable for field applications during the oak wilt season.

FINDINGS AND IMPLICATIONS

The developed colorimetric LAMP kit demonstrated a high sensitivity of 100 percent and a specificity of 90 percent when tested on 39 oak tree samples, indicating its robustness in detecting *Bretziella fagacearum*. The rapid detection capability, with visual confirmation within 25 minutes and a total testing time of under 45 minutes, makes this method highly efficient for large-scale screening during the oak wilt season. The simplified DNA extraction protocol, requiring only 20 minutes, further enhances the practicality of this testing approach in field conditions.

The freeze-dried LAMP reaction mixture and primers allow for easy transport and minimal setup, reducing potential errors during on-site testing. This portable and user-friendly diagnostic tool has significant implications for managing and controlling the spread of oak wilt. By enabling early detection and timely removal of infected trees, this method can help prevent the widespread damage caused by the disease and protect healthy oak populations. The success of this LAMP-based detection platform paves the way for its application in other plant disease diagnostics, offering a valuable tool for both researchers and forestry professionals.

LITERATURE CITED

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