LAMP as a tool for rapid identification of subterranean oak roots

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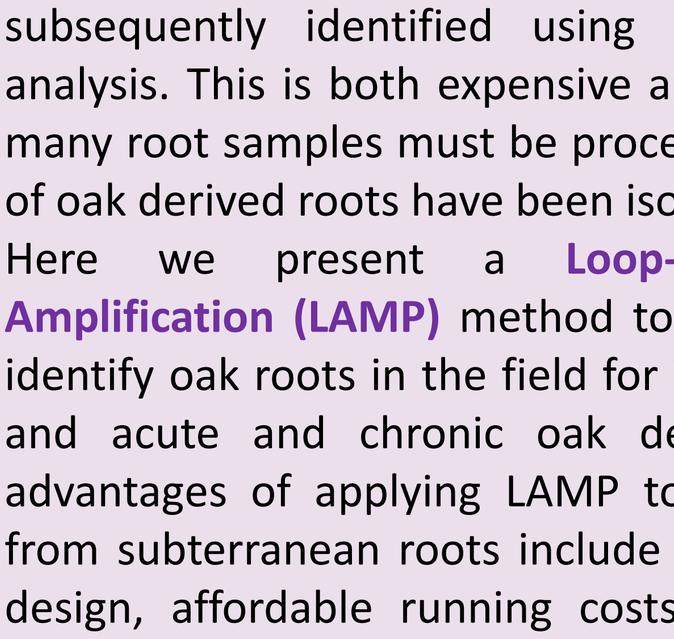
Introduction

Acute and chronic oak decline, specifically pedunculate (*Quercus robur* L.) and sessile (*Q. petraea* (Matt.) Liebl.) oak, has become of great concern to the United Kingdom and Europe in the last few years (1). To understand the relevant causative agents of acute and chronic oak decline and define the progression of the syndromes, an integrated approach has been adopted, and includes monitoring of soil condition, climate changes, insect populations, microbial landscaping and possible genetic predisposition.

Sampling of oak trees themselves is relatively easy for arboreal leaves and stems as the trees can easily be visually identified. This is not the case for subterranean (rhizosphere) sampling of roots where oak trees grow side by side many other plant and tree species. Oak roots do not display any obvious visual identifiable traits to permit easy identification and separation of different tree and plant species during sampling. Currently samples must be returned to laboratory, DNA extracted, and tree species subsequently identified using PCR and/or sequence analysis. This is both expensive and time consuming and many root samples must be processed to ensure a subset of oak derived roots have been isolated.

Here we present a **Loop-Mediated Isothermal Amplification (LAMP)** method to rapidly distinguish and identify oak roots in the field for improved root sampling and acute and chronic oak decline monitoring. The advantages of applying LAMP to identify plant species from subterranean roots include its simple experimental design, affordable running costs, and readily available reagents and portable equipment.



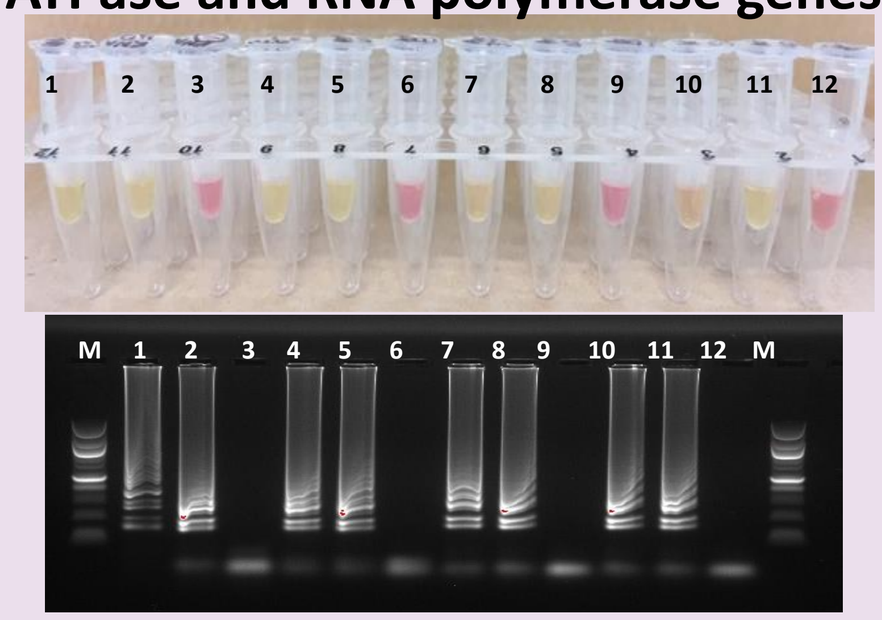
An overview of Loop-Mediated Isothermal Amplification (LAMP).

LAMP uses two sets of primers and a polymerase with high displacement and replication activity (BstI) to amplify the target sequence at a constant temperature of 65°C. The reaction produces more product than a typical PCR reaction. Picture credit (2)

Literature cited

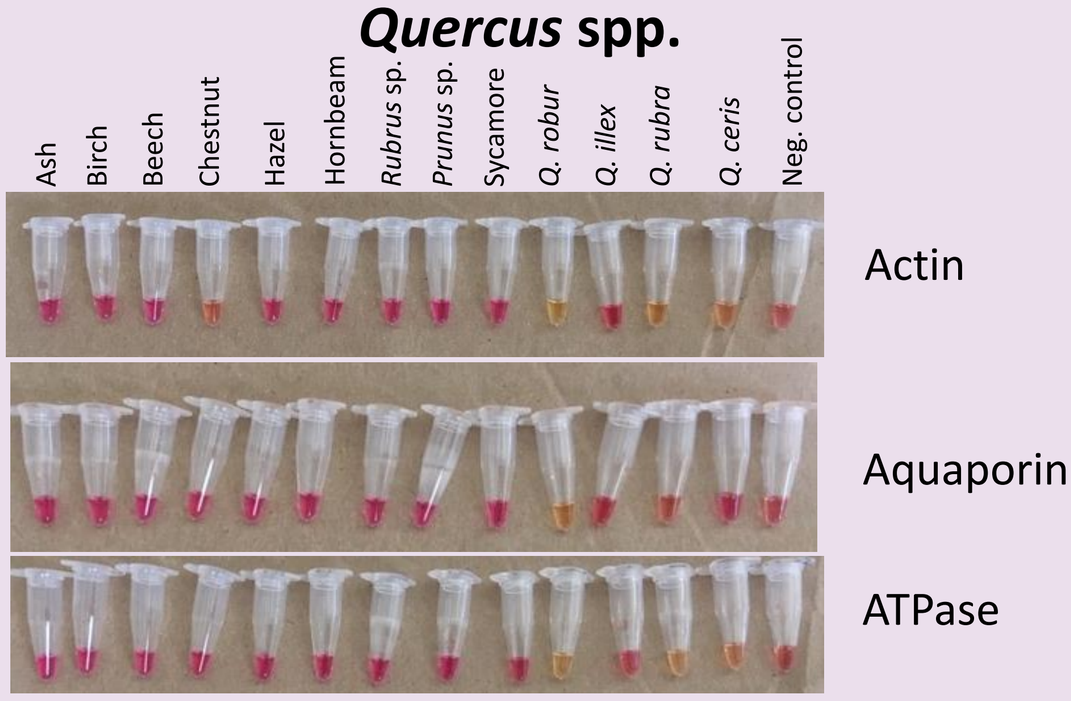
1. Brown N et al., 2018. Predisposition of forests to biotic disturbance: Predicting the distribution of Acute Oak Decline using environmental factors. *Forest Ecology and Management* **407**, 145-54.
2. Lmstanfield at English Wikipedia, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=59313952

LAMP of *Quercus robur* actin, aquaporin, H+ ATPase and RNA polymerase genes



Colorimetric LAMP (A) and subsequent agarose gel electrophoresis (B) of actin, aquaporin, H+ ATPase and RNA polymerase genes from oak genomic DNA isolated from fine roots. Tubes/Lanes 1 and 2, LAMP amplification of actin gene; tube/lane 3, actin negative control; tubes/lanes 4 and 5, LAMP amplification of the aquaporin gene; tube/lane 6, aquaporin gene negative control; tubes/lanes 7 and 8, LAMP amplification of the H+ ATPase gene; tube/lane 9, H+ ATPase negative control; tubes/lanes 10 and 11, LAMP amplification of RNA polymerase gene; tube/lane 12, RNA polymerase negative control. Reactions that were scored as positive in the colorimetric test (change from pink to yellow) yielded positive results when run on an agarose gel. Reactions that were pink in colour exhibited no amplification. Lane M, 100 bp marker (New England Biolabs).

Specificity of LAMP assays for detection of *Quercus* spp.



Colorimetric LAMP of DNA from various forest species to test the specificity of each of the gene primer sets for *Quercus* spp. LAMP positive reactions change colour from pink to yellow due to the presence of an indicator dye that detects the release of protons and an accompanying pH change during PCR amplification.

Conclusions

- We have successfully applied LAMP in the laboratory to detect *Quercus* sp. fine roots.
- All four genes tested were successfully amplified from *Q. robur.*

Future direction:

- Validate assays with biological replicates of forest tree species.
- Field test DNA extractions and run LAMP on a Genie II platform in the field.