PRACTICE ABSTRACT

# Quantification of arbuscular mycorrhizal fungi by quantitative polymerase chain reaction

## Problem

Arbuscular mycorrhizal fungal (AMF) root colonization is traditionally measured by microscopy. Roots are first stained and then carefully mounted on a glass slide before examination on the microscope to identify and count fungal structures inside the roots. But microscopy is labor-intensive, and results depend on the observer.

## Solution

Methods such as, quantitative polymerase chain reaction (qPCR) can improve quantification and analysis of AMF. This practice abstract provides a short protocol describing qPCR as a method to quantify AMF in plant roots.

## Benefits

The qPCR presents a reliable alternative method to quantify AMF root colonization that is less opera-tor-dependent than traditional microscopy and offers scalability to high-throughput analyses. Advantages over microscopy are:

- Larger datasets can be analysed/quantified more thoroughly, in a faster amount of time
- qPCR provides more accurate and precise detection on amplified DNA sequences
- qPCR is highly specific and sensitive as opposed to visual observations made using microscopy
- why important for solace: to compare novel genotypes in their efficiency to form mycorrhizal symbiosis

## Definitions box

**Arbuscular mycorrhizal fungi (AMF)**

AMF are a group of soil microorganisms that form mutualistic symbiosis with most terrestrial plants. This mutualistic symbiosis can benefit water and nutrient supply to the host plant, improving growth.

**Polymerase chain reaction (PCR)**

A now widely used scientific method to rapidly copy specific DNA samples, enabling scientists to amplify small samples of DNA to a large enough amount to study in detail.

**Ribonucleic acid (RNA)**

A nucleic acid essential for metabolic processes to produce proteins in all living cells. RNA carries genetic information and can be formed in varying lengths and sizes.

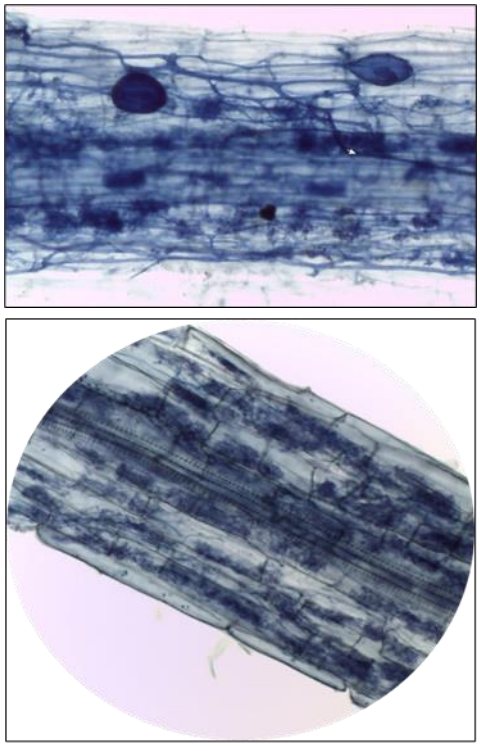

**Figure 1. Maize (top) and tomato (down) roots colonized with AMF (Sarah Symanczik and Tabea Gallusser, FiBL)**

## Practical recommendation

- Our proposed qPCR method is based on relative quantification, where the levels of the AMF gene is normalized with a plant gene.
- The AMF community is quantified based on the 18S rRNA gene fragment, amplified with the primers AMG1F (5'- ATA GGG ATA GTT GGG GGC AT -3') and AM1 (5'- GTT TCC CGT AAG GCG CCG AA -3') as described by Hewins et al. (2015).
- We designed the PCR primers GAP_f1 (5'-TGG AAT GGC CTT CAG AGT TC-3') and GAP_r3 (5'-TCT GTG GAA ACC ACA TCG TC-3') to amplify the glyceraldehyde-3-phosphate dehydrogenase gene for petunia. For other plant species, primers need to be chosen based on the literature or the genome sequences if available. Any conserved sequence can be used as a target sequence.

- Sample preparation: Roots are carefully washed to remove adhering soil and dried with clean tissue. Fine roots are separated, cut into small pieces and stored at -20°C until DNA extraction.
- DNA extraction: DNA is extracted from grounded lyophilized roots following the recommendations of the DNA extraction kit e.g. NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany).
- DNA quantification: DNA can be quantified via spectrophotometry or fluorescence in order to dilute DNA extracts to approx. 10 ng/µL DNA.
- qPCR reaction: Reactions are carried out in 20 µL containing onefold HOT FIREPol EvaGreen qPCR Mix Plus (Solis Biodyne, Tartu, Estonia) or any other DNA polymerase qPCR mix, 250 nM of each primer, 0.3% bovine serine albumin, approximately 10 ng of root DNA. Samples are analysed in three technical replicates (triplicates) and each qPCR run includes no-template controls
- qPCR cycling conditions: the qPCR program consists of an initial denaturation step of 15 min at 95 °C, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 62 °C for 30 s, and elongation at 72 °C for 20 s followed by a melting curve analysis (from 65 to 95°C, with 0.5 °C steps holding for 5 s). Cycling conditions might vary depending on the chosen DNA polymerase qPCR mix.

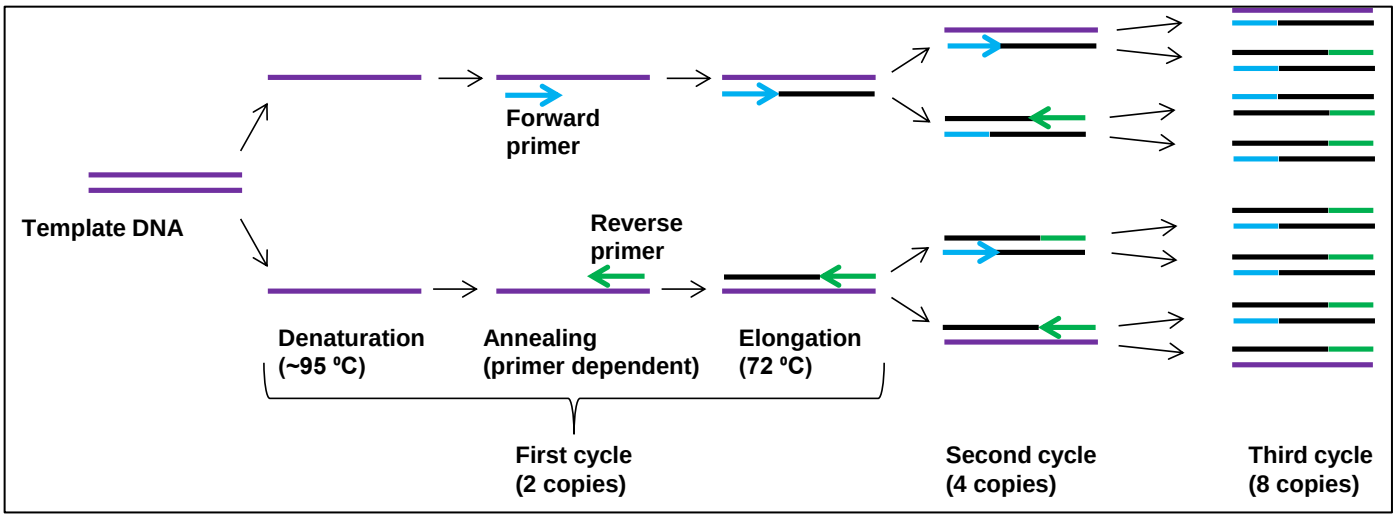


**Figure 2. The exponential amplification of DNA in PCR (Sarah Symanczik, FiBL).**

## Further information

### Further readings


- Bodenhausen, N., Deslandes-Hérold, G., Waelchli, J. et al. Relative qPCR to quantify colonization of plant roots by arbuscular mycorrhizal fungi. Mycorrhiza 31, 137–148 (2021). https://doi.org/10.1007/s00572-020-01014-1
- Hewins CR, Carrino-Kyker SR, Burke DJ (2015) Seasonal variation in mycorrhizal fungi colonizing roots of Allium tricoccum (wild leek) in a mature mixed hardwood forest Mycorrhiza 1–15 https:// doi.org/10.1007/s00572-015-0628-5


## About this practice abstract and SolACE


**Publisher:**
Research Institute of Organic Agriculture FiBL
Ackerstrasse 113, Postfach 219, CH-5070 Frick
Phone +41 62 865 72 72, info.suisse@fibl.org, www.fibl.org



**Authors:** Sarah Symanczik and Natacha Bodenhausen

**Contact:** sarah.symanczik@fibl.org, natacha.bodenhausen@fibl.org



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SolACE: The project is running from May 2017 to April 2022**.** The goal of SolACE (Solutions for improving Agroecosystem and Crop Efficiency for water and nutrient use) is to help European agriculture face major challenges, notably increased rainfall variability and reduced use of N and P fertilizers

**Project website:** www.solace-eu.net




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