Root2Res
Root phenotyping and genetic improvement for rotational crops resilient to environmental change

Practice Abstract

Absolute qPCR quantification of arbuscular mycorrhiza fungi in crop plants

Problem

Conventional methods to quantify arbuscular mycorrhizal fungal (AMF) colonization include microscopy, which involves staining of the roots and meticulous observation. This method is time consuming and subjective because it depends on the observer's level of expertise.

Applicability box

Theme: qPCR, mycorrhiza, quantification

Relevance: AMF-plant symbiosis has been shown to increase plant health.

Best in: Wheat and tomato

Solution

The use of broad range qPCR primers was validated to quantify AMF root colonization in different crops. This high-throughput and reliable technique is versatile and suitable for a wide range of research projects.

Benefits and weaknesses

Compared to the conventional microscopy method, qPCR allows the simultaneous analysis of a larger number of samples. Moreover, the results of qPCR are not influenced by observer subjectivity. AMF quantification can be complemented by sequencing the PCR product to assess AMF diversity. One weakness of the qPCR method is that it cannot distinguish between the different AMF structures. In addition, it is worth noting that the qPCR method yielded inconclusive results when applied to leek, possibility due to negligible changes in colonization rate (Corona Ramírez et al. 2023). We conclude that the qPCR method should be validated for each crop.

Practical recommendations (figure 1)

1. Order 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.

2. Prepare a standard using DNA of a single AMF species or environmental sample following the manufacturer's recommendation of a cloning kit.

3. Collect and wash the plant roots and store in the freezer.

4. Grind the roots, for example by placing them in a 2 ml reaction with two 5 mm metal beads, freeze for 2 h at -80 °C, and grind with the TissueLyser II (Qiagen).

5. Weigh the exact amount of ground roots and extract the DNA, for example with the kit DNeasy® Plant Mini Kit (Qiagen).

6. Quantify the DNA concentration and dilute, if necessary, with molecular grade water.

7. Prepare the qPCR reaction (final volume per sample 15 µl): 7.5 µl of KAPA SYBR FAST qPCR Master Mix 2x (or other), 250 nM of each primer and 1.5 µl of DNA template. Each sample is measured in triplicates. Include a dilution series of the standard as well as a negative control.

8. qPCR program: initial denaturation 3 min at 95 °C, 40 cycles of denaturation at 95 °C for 10 sec, annealing at 62 °C for 30 sec, and elongation at 72 °C for 20 sec and melting curve from 55 to 95 °C. Program needs to be adjusted if using a different SYBR mix.

9. Using the standard, a regression line is calculated to determine the number of gene copies present in each sample, for example using the program which goes with the qPCR machine.

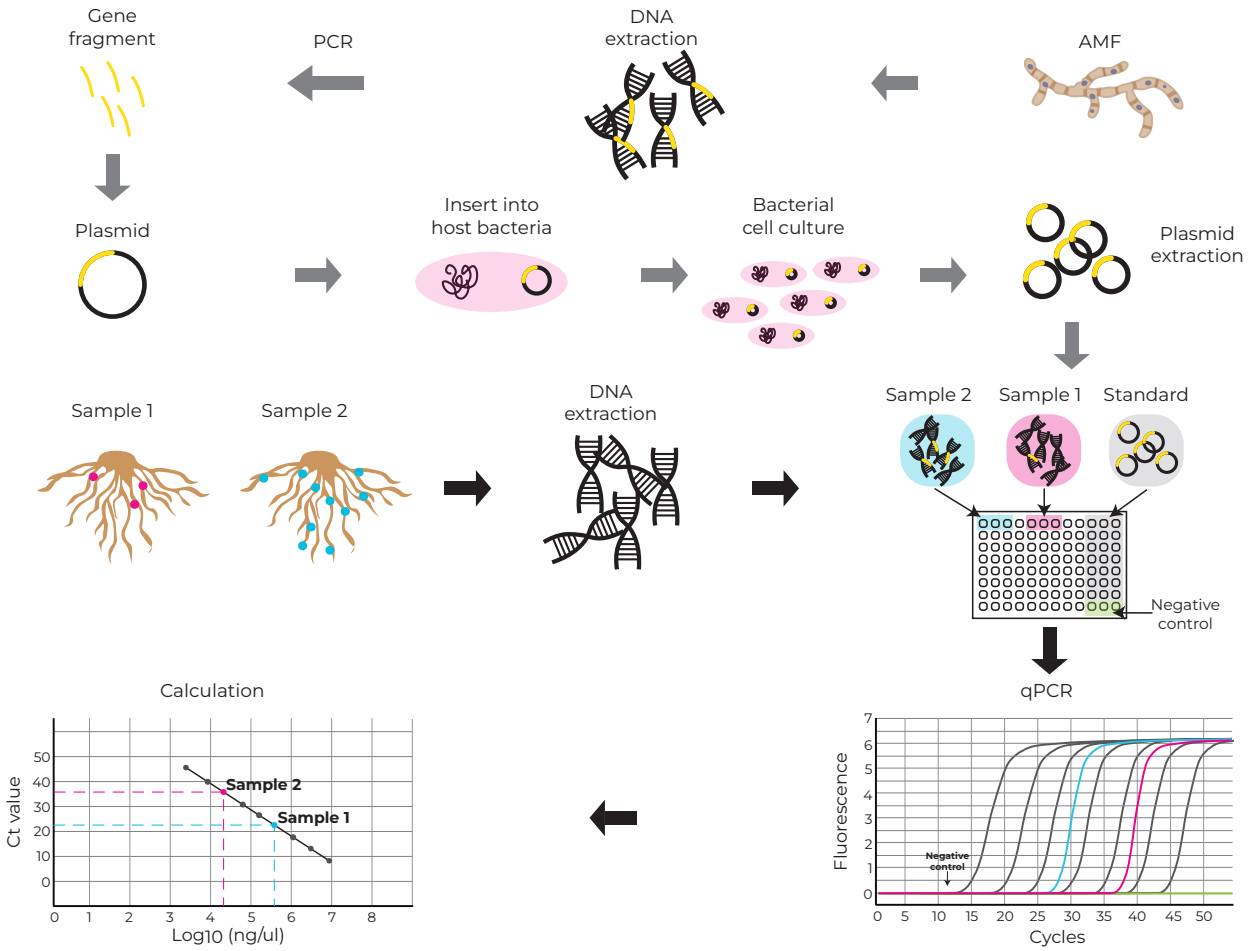

Figure 1. Graphical representation of the steps for the preparation of the standard and the quantification of AMF with qPCR. Graphic: Andrea Corona Ramirez, 2023

Further information

- Corona Ramírez A, Symanczik S, Gallusser T, Bodenhausen N. (2023). Quantification of arbuscular mycorrhizal fungi root colonization in wheat, tomato and leek using absolute qPCR. Mycorrhiza. https://doi.org/10.1007/s00572-023-01122-8
- 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. https://doi.org/10.1007/s00572-015-0628-5

About this practice abstract and Root2Resilience

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Root2Resilience: The project is running from September 2022 to August 2027. The overall goal of Root2Resilience – Root phenotyping and genetic improvement for rotational crops resilient to environmental change – is too develop root phenotyping, genetic and modelling tools and use them to define and test innovative genotype ideotypes able to enhance the tolerance to abiotic stress and carbon sequestration in soils

Project website: root2res.eu

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