Visualization of Mycorrhizal Fungi

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Introduction

Two major roles of fungi are emphasized in most introductory biology courses. First, students learn that many fungi are decomposers and, thus, facilitate recycling of nutrients. Second, students learn that some fungi are virulent pathogens. However, the important role that fungi play as symbionts is often not stressed.

Mycorrhizae (Allen, 1992; Harrison, 1997; Smith and Read, 1997) are mutually beneficial intimate relationships between diverse fungi and approximately 90% of plants. The exact nature of the relationship and the morphology of the fungus depend on the plant, the fungus, and other factors such as plant developmental stage and nutrient status. Commonly, the fungus surrounds and penetrates roots, but the fungus does not penetrate the plant-cell membrane. The roots provide a carbon source for the fungus. Meanwhile, the fungus may extract nutrients from a large volume of soil and deliver them to the plant, or the fungus may confer protection against biotic and abiotic stresses.

A laboratory exercise that complements a lecture on mycorrhizae provides important reinforcement. Fortunately, it is relatively easy and inexpensive to stain only fungi (Phillips and Hayman, 1970) in and on roots. The presented protocol may be used simply as a demonstration exercise, or it may provide the experimental basis for investigating open-ended questions that students pose.

Personal Equipment (for each student)

- Personal safety equipment (steps 1-8)
- Razor blade, 1 (step 1)
- 25-ml Pyrex beaker, 3 (steps 1-8)
- Forceps, 1 (steps 1-9)
- Pasteur pipette and bulb, 1 (step 3)
- Beaker tongs, 1 (steps 2 & 3)
- Petri dish, 1 (step 4)

General Equipment (for class of 20 students)

- Water Bath, 2 (steps 2 & 6)
- Microscope, 40× overall magnification, 5 (step 9)

Reagents (for class of 20 students)

Commercial Reagents to Purchase:

- 10% (w/v) KOH, 300 ml (steps 2 & 3)
- 30% H_2O_2, 1 ml (step 3)
- 10% (v/v) HCl, 300 ml (step 5)
- 85% (w/w) DL-lactic acid, 600 ml (steps 6-8)
- aniline blue, 150 mg (step 7)

Custom Reagent to Prepare:

- 0.05% (w/v) aniline blue/lactic acid solution (step 6) (150 mg aniline blue added to 300 ml 85% (w/w) DL-lactic acid)

Notes for the Instructor

Choice of Roots. Tender roots require a minimum incubation period (step 2), whereas woody roots require a more stringent incubation period. The incubation period and the incubation temperature must be optimized for each species. As examples, we have used the following times with good results: Bahia grass (*Paspalum notatum*), 10 minutes; pawpaw (*Asimina triloba*), 60 minutes; trifoliate orange (*Poncirus trifoliata*), fig (*Ficus carica*), live oak (*Quercus virginiana*) and spruce pine (*Pinus glabra*), 120 minutes, all at 80°C. With tender roots, incubation at room temperature is sufficient. Finer roots lend themselves better to microscopy (step 9).

Explanation of Protocol. The purpose of the KOH-heating step (step 2) is to remove pigments. The KOH solution must be changed if it becomes dark. The peroxide step (step 3) can be skipped if roots are completely decolorized by the KOH incubation. As a matter of simplicity, however, we have always included the peroxide treatment with no deleterious results.

Our experience indicates that stained roots can be stored (step 8) for up to several weeks before microscopic examination.

Safety. Some procedures employ dangerous reagents, sharp objects, and high temperature. Appropriate safety precautions should be taken.

Supplementary Information. A 25-MB PowerPoint 97 Presentation of the procedure and example results are available at an anonymous ftp site (Computer IP: 128.186.14.75; d:/data/public access—read only).

Student Outline

1. Select and rinse roots ≤ 2 mm in diameter. Cut roots to about 3 cm in length.
2. Place roots in a beaker. Add 10 ml 10% KOH (enough to cover roots). Transfer the beaker to an 80°C water bath and incubate for 15 to 120 minutes, as directed by your instructor.
3. Remove the beaker from the water bath with tongs. Add a drop of 30% H_2O_2 to the KOH solution that contains the roots (i.e., ~40 µl H_2O_2/10 ml KOH). Incubate for 10 minutes at room temperature.
4. Transfer roots to a Petri dish. Rinse roots for 15 seconds with tap water at room temperature. Repeat the rinse 3×.
5. Transfer roots to a beaker containing 10 ml 10% HCl (enough to cover roots). Incubate for 5 minutes at room temperature.
6. Transfer roots directly from the 10% HCl into a beaker containing 10 ml 0.05% aniline blue/lactic acid solution (enough to cover roots). Transfer beaker to 80°C water bath and incubate for 30 minutes.
7. Transfer roots from the aniline blue/lactic acid solution to 10 ml 85% lactic acid. Incubate at room temperature for 5 minutes.
8. Store roots in 85% lactic acid.
9. Wet-mount stained roots and examine microscopically. Fungi are stained blue; root tissue is not stained.

References

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