

Clearing and Staining of Endomycorrhizal Roots 11/21/2006

Supplies needed:

- Gloves
- Lab coat
- Histosette cassettes (we use cassettes as seen in Fisher catalog [cat. #15-182-500A])
- Sieve for washing roots
- Drying oven
- 3 large beakers (1000mL)
- Graduated cylinders
- Heating plate
- 2.5 % KOH
- 1% HCl (acidified water)
- 0.05 % Trypan blue stain solution
- De-stain solution (acidified glycerol; 500 ml Glycerin (= Glycerol) + 450 ml distilled H₂O + 50 ml 1% HCl)
- 3% Hydrogen peroxide

Instructions:

1. If roots are dried, allow to re-hydrate for 1-2 hours in water.
2. Wash roots with tap water on a sieve then transfer some of the roots into a Histosette cassette.
*Wear gloves, safety glasses and a lab coat for remaining steps.
3. Clear roots in Histosettes by soaking them in 2.5 % KOH overnight, or by soaking them for 20-30 minutes in the heated KOH solution at 90 °C.
NOTE: Check for color change to light brown then remove from heat. Do not over-clear; cortex of roots will disintegrate. Different root types will require different clearing times and intensities. Fine herbaceous roots will require much less time (15-20 minutes). To minimize the time in KOH preheat the KOH before adding cassettes.
4. Pour off KOH in a waste bottle, and rinse Histosettes in DI water 2-3 times.
5. To bleach root pigments place Histosettes in a solution of alkaline H₂O₂ for 10-30 minutes (see below for recipe). This bleaching step is only necessary for pigmented roots. Not necessary for herbaceous roots.
 - a. Mix a fresh solution of alkaline 3% H₂O₂ just prior to usage.
 - b. Mix and perform bleaching under hood.
 - c. Preheat solution on heating block until it has just begun to boil. Turn down heat to prevent excessive foaming and boiling over.
 - d. Place Histosettes in solution. Stir occasionally. Check roots periodically. Remove when roots are white or yellow/white in color.
 - e. Pour off H₂O₂ into a waste bottle, and rinse again in DI water 2-3 times.
6. Acidify roots for 10 minutes in 1% HCl at room temperature. This is necessary for trypan blue stain to bind to fungal structures.
7. Pour off HCl in a waste bottle, and remove Histosettes from HCl and place in beaker with 0.05 % trypan blue stain. Place the solution in drying oven at 90 °C for 30-60 minutes or on hot plate at 90 °C for 5-20 minutes or leave it overnight.

8. Pour off trypan blue solution in a waste bottle, and place Histosettes in de-stain solution for a few days.
**If roots are not bleaching in alkaline H₂O₂ after considerable time or the trypan blue stain did not take, they were probably not cleared enough. You can start over by placing cassettes in 10% KOH. However, be aware that KOH is very destructive to the roots and if left in too long, the cortex will be stripped.
9. For permanent mounting, refer to the web site below:
<http://invam.caf.wvu.edu/methods/recipes.htm>

References:

A modified procedure for staining roots to detect VA mycorrhizas
RE Koske, JN Gemma Mycological Research 92:44, 486-488, 1989.

Quantification of vesicular-arbuscular mycorrhizae in plant roots.
PP Kormanik, AC McGraw 1982. K 03096 Mycorrhiza;