STAINING ROOTS FOR ARBUSCULAR MYCORRHIZAL FUNGI

Materials
- tissue cassettes
- DI water
- squeeze bottle
- 2.5% (w/v) potassium hydroxide
- 0.6% sodium hypochlorite (Clorox diluted 10x)
- 1% hydrochloric acid
- trypan blue
- reagent bottles
- drying oven

Methods
1. Prepare acidic glycerol and trypan blue solutions:
   Acidic glycerol/trypan blue:
   - 500 mL glycerol
   - 450 mL DI water
   - 50 mL 1% HCl
   - 0.5 g trypan blue
   Acidic glycerol:
   - same as above, omitting trypan blue
2. In sieve or tissue cassettes, rinse roots 3x in DI water.
3. Heat roots (in cassettes) in 2.5% KOH 10-30 min @ 90°C.
4. Rinse roots 3x in DI water.
5. Bleach dark roots in 0.6% NaOCl 10-30 min @ 90°C. Rinse 3x in DI water.
6. Soak roots in 20-50 vol. 1% HCl for 1-24 hours (e.g., overnight) to pH 0.8-1.4.
7. Soak roots in acidic glycerol/trypan blue for 10-30 min @ 90°C.
8. Destain and store in acidic glycerol. Keep staining solution for reuse; store in a dark bottle. Trypan blue solutions require special disposal; retain all waste and call EHRS for pickup.

Reference: