POT CULTURING

INTRODUCTION

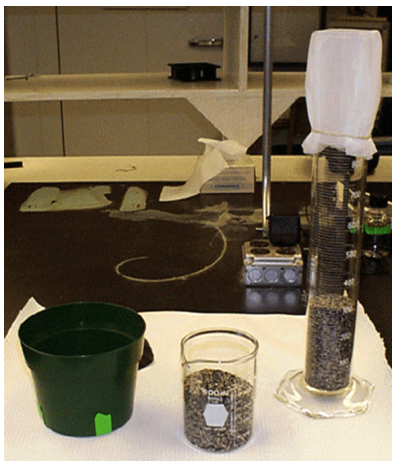
Pot culturing procedures can vary greatly, depending on what your starting material is and what you want to have in the end. If you are starting with field soil, methods can be found at the INVAM website to obtain monospecfic cultures successfully (http://invam.caf.wuv.edu). If you wish to use mixed population cultures for your glomalin studies, you may do this by following similar methods. In measuring glomalin production from field inoculum or under various conditions, it is inadvisable to use field soil since this contains a background concentration of glomalin that will make your results inconsistent. For these experiments (where a hyphal compartment is made), rinsed spores and/or hyphae collected via wet sieving from pot cultures, ordered from INVAM or from field samples may be used. (Note: If you wish to use field samples, it is advised to follow the procedures detailed from INVAM to obtain monospecific or mixed cultures and a large amount of viable inoculum.)

MATERIALS

Rinsed spores and/or hyphae from previous pot cultures or from INVAM
2:1 coarse:fine sand mix or 1:1 sand:coal waste mix
20 mM sodium citrate, pH 7.0
6” pots
half-strength Hoagland’s solution (see the following)
automatic watering system (optional, see the following)
40 um nylon mesh
black horticultural mesh (weed block)
1000 ml graduated cylinder
cup or 400 ml beaker
rubber bands

METHODS

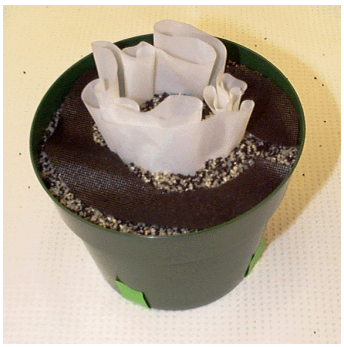
(1) Cut horticultural mesh into squares that can fit in the bottom of the pots and block bottom holes and into rings, about 1-1.5”thick, to fit on top of the sand and around the nylon mesh bag (These rings aid in water distribution and in controlling algal growth.)
(2) Cut nylon mesh into 10-11”diameter circles (1/pot) and into 3x6 cm strips (4-6/pot) (strips are optional)
(3) Sterilize pots, graduated cylinder, cup or beaker, and mesh squares, rings, circles, and strips in ~10% bleach for at least 1 hr, rinse thoroughly with distilled water and dry covered
(4) Extract residual glomalin from sand by covering with 50 mM sodium citrate, pH 8.0, and autoclaving for 1 hr at 121^{o}C
(5) Remove citrate solution and wash thoroughly with distilled water
(6) Sterilize sand and coal waste (if you are using this) by autoclaving for 1 hr at 121^{o}C
(7) Cover side holes in pots with labeling tape, place mesh square on bottom to cover bottom holes
(8) Place ~300 mls of sand mix or sand:coal waste mix in graduated cylinder and center the nylon mesh circle over the top of the cylinder, secure with a rubber band near the top of the mesh



(9) Flip cylinder over into center of pot and fill around it with sand mix or sand:coal waste mix using the cup or beaker

(10) Release rubber band and carefully pull out the cylinder (nylon mesh should have formed a bag that will separate the plant roots from the surrounding media, forming a root/hyphal compartment and a hyphal compartment)

(11) Place nylon mesh strips in the surrounding media, outside of the mesh bag, to make periodic measurements of hyphal growth and glomalin accumulation (optional)
(12) Place mesh ring on top of the media outside of the mesh bag



(13) Make a hole in the center of the media in the mesh bag, ~1” deep, add about 12 sudangrass seeds (or whatever host you are using (the number of seeds can also vary with host)) and the spores and/or hyphae and cover
(14) Water with half-strength Hoagland’s solution, either by using an automatic watering system or by watering over the mesh ring by hand
(15) Harvest after 3-4 months by carefully removing the mesh bag and storing the material from the mesh bag and the surrounding media separately, perform protein extraction, percentage colonization, hyphal measurements, or other analyses on the different fractions as desired (Refer to Wright, Franke-Snyder, and Morton, 1996 and Wright and Upadhyaya, 1996 to see what measurements are typically made.)

HOAGLAND'S NUTRIENT SOLUTION (Millner & Kitt, 1992)

Nutrient	g/500ml stocks	stock concen.	mls of stock/L solution	final concen.
$Ca(NO_3)_2*4H_2O$	118.1	1 M	2.5	2.5 mM
KNO_3	50.55	1 M	2.5	2.5 mM
$MgSO_4*7H_2O$	123.24	1 M	1	1.0 mM
KH_2PO_4	6.81	0.1 M	0.2	20 uM
NaFeEDTA	1.84	0.01 M	5	50 uM
$Na_2MoO_4*2H_2O*$	0.24	0.002 M	0.1	0.2 uM
H_2BO_3*	3.09	0.01 M	0.1	10.0 uM
$NiSO_4*6H_2O*$	0.26	0.002 M	0.1	0.2 uM
$ZnSO_4*7H_2O*$	1.44	0.01 M	0.1	1.0 uM
$MnCl_2*4\ H_2O*$	1.98	0.02 M	0.1	2.0 uM
$CuSO_4*5\ H_2O*$	0.62	0.005 M	0.1	0.5 uM
$CoCl_2*6\ H_2O*$	0.24	0.002 M	0.1	0.2 uM
MES	19.525	0.2	2.78	

* These are micronutrients and can be made up together. Smaller amounts of the stocks can be made up for small or short experiments, because the stocks can get contaminated (especially the potassium phosphate stock).

AUTOMATIC WATERING SYSTEM

(Millner & Kitt, 1992)

INTRODUCTION

An automatic watering system can be designed that will reduce the amount of maintenance required to grow these plants for 3-4 months. It may also help in mycorrhizal production and plant health by watering the pots several times a day (typically 4 times), which will keep the media constantly moist and will reduce wet/dry cycles that may reduce hyphal production. Most of the materials can be obtained from horticultural or gardening supply stores or in the gardening sections at Walmart or Target. It is best if all of the tubing and connectors are black to prevent algal growth.

MATERIALS

32 gallon rubber garbage can, on wheels and with a lid
7.5" cable ties (Cat-Pak®)
solid ¼" rubber, drip tubing (Moisture Master™) (feeder tubes)
¼" porous mini soaker hose (Moisture Master™)
circulating pump (~120 VAC, 60 Hz)
½" solid, rubber tubing (mains and pump connector)
plastic 2- and 3-way connectors for ¼" tubing
appropriate connectors for connecting the ½" tubing to the pump
wooden frame or suspension device for main lines and feeder tubes
screw connectors from ½" tubing from pump to mains (for easy disconnection and nutrient refill)
flow check valves
plugs for ½" tubing
cork borer
electrical timers

METHODS

(1) Sterilize all tubing in 10% or stronger bleach solution for 1 hr and rinse thoroughly with distilled water
(2) Make up half-strength Hoagland's solution in the garbage can, as directed. Make enough for 36 L (do not make up more than 48 L, because you want the solution to cycle thru rapidly enough to prevent microbial growth).
(3) Connect ½" tubing to the pump (make tubing long enough for the pump to reach the bottom of the can and reach about 6" above the can)
(4) Place screw connectors and a check valve on the end of the tubing from the pump and connect ½" tubing (enough for it to reach the end of a row of pots and be suspended about 1-2 ft above the pots)
(5) Place wooden frame over the pots (making a bar behind the pots about 1-2 ft above them) and tie the ½" tubing to the bar with the cable ties (forming mains)
(6) Make 3-4" diameter rings using the ¼" porous tubing and the 3-way connectors (if you are using Deepots, the rings should be even smaller)
(7) Make holes in the ½" tubing with a cork borer and fit 2-way connectors in the holes
(8) Connect the ¼" feeder tubes to the mains via the 2-way connectors and to the rings via the 3- way connectors (make sure that the feeder line is not much longer than the distance to the pot, so it doesn't sag)
(9) Suspend the rings above the pots using the cable ties to make a tri-pod that gets taped to the outside of the pot
(10) Plug the power source for the pump into a timer that is set to run the pump for 20-30 sec, 4 or more times a day (when the lights are on)