Staining Arbuscular Mycorrhizae Lab

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INTRODUCTION: Arbuscular mycorrhizal fungi (AMF) are well known to have important impacts on plant developments in nutrient poor soils. Plants colonized by AMF grow better, have better root disease resistance, and better water acquisition than those that are not. All of this comes at a high price, as plants can spend as much as 20% of their photosynthates on AMF. The mutualism, however, is fragile and as soon as plants have enough nutrients in the soil, they kick out AMF to not waste their photosynthates on feeding an unnecessary symbiont. The result is that in overfertilized soils, AMF cannot be found in roots.

The system root is a living entity and as it grows and expands, new roots are formed and old roots die. Newly formed roots are open niche space for microbes to colonize. Nutrient availability, competitors, and the strength of association with a particular plant will determine how much of that root system is colonized. Knowing how much of the root system is colonized by AMF can give you a lot of information such as how "good" a symbiont is with their host.

OBJECTIVE

We will observe AMF today under the microscope. You will learn how to count % root colonized using the gridline intersect method.

MATERIALS

Root of green onions and sorghum, inoculated with a single AMF species and grown for 2 months
10% KOH
10% Lactic acid
Acid fuchsin stain
Destaining solution
Slides
Coverglass
Forceps
gridded paper and scissors
Petri plates
Acid collection container
KOH collection container

METHODS

1. Pick out one sorghum and one green onion plant. Wash the root system to clean off most of the growing media.
2. Cut the root system into 1 cm pieces, place into container and add enough 10% KOH to cover them. Incubate at room temperature for at least 12 hours to clear the roots of cellular content.
3. Drain the stained roots of 10% KOH, and rinse in water at least 3X. At this stage the roots are fragile so be gentle with them.
4. Add 10% lactic acid to just barely covering the roots and warm the beaker in a water bath if available. The liquid only needs to be warmed up. Do not boil the acid solution.
5. Allow the sample to rest for 3 minutes. Drain the roots of the 10% lactic acid.

6. Add a little of the Acid Fuchsin stain solution to cover the roots. You don't need much.
7. Warm the solution up using either a water bath or quickly microwaving without boiling the solution. In class, we will allow this solution to sit for 5 minutes with constant warming and swirling. For best results, allow the roots to sit for at least 30 min or if not warmed, the roots may be stained for 12-24 hours.
8. Use dissecting needles or forceps to gently grab the roots and place them on several folds of papers to help drain off excess stain. Pour the used stain into the appropriate container.
9. Place the drained roots into a different cup with a some destaining solution. Agitate gently to remove the excess stain. Repeat once more. Pour used destaining solution into appropriate containers.
10. Set up your petri plate by placing a gridded piece of paper inside the lid of a petri plate. Place the bottom of the petri plate into that lid. Your petri plates now have gridlines.
11. Pour a little bit of unused staining solution into the dish and place 5 root pieces randomly into the liquid. You will observe all of them for colonization.
12. Before picking out each piece of root for observation, count the number of lines (both vertical and horizontal) that each piece intersects and record that number for that particular piece of root.
13. Place the root onto a microscope slide and place a cover slip on top to make a wet mount. Observe under a 40X objective for colonization. If anywhere on this piece of root has AMF colonization, you would consider all the lines that they intersect as colonized; likewise for non-colonized roots.
14. Calculate the total length of root using the following formula:

$$R = \frac{\pi A n}{2H}$$

R = total root length
π = 3.14156
A = area in which roots are distributed (65.61 cm^2)
n = number of root-gridline intersections
H = total length of straight lines (133 cm)

15. Use the formula to calculate the total root length that is colonized and not colonized.
16. Take any photos you need for your report.

Consider these questions: Which plant species has better colonization by this AMF species? Would you expect that the AMF would provide the same benefit to each plant species? Why or why not?