Medicago truncatula Seed Extraction and Germination

I. Seed Extraction (Blender Method)

By briefly pulsing the pods in a blender the seed remain undamaged, resulting in close to 100% germination.

1. Put the gray pods in a Waring blender (the large glass holder works much better than either the small metal cup or the small plastic beaker like holders).

2. Pulse for 1-2 seconds.

3. Pour the pods and loose seeds (auburn crescent shaped) onto a 2.00mm/0.85mm stacked laboratory test sieve.

The 2.00mm sieve allows the loose seeds through but retains the pods, and a 0.85mm sieve retains the seeds but lets powder debris through.

4. Pour the pods that did not pass through the 2.00mm sieve back into the blender.

5. Pulse for 1-2 seconds.

6. Pass this through the stacked sieves.

7. Continue this process until there are no more seeds being extracted; it's an iterative exercise of blending, straining, putting pods back into the blender, pulsing again, etc. About 100 pods require about 25 iterations.

II. Surface sterilization and germination of seeds

Seeds need to be scarified in order to break the seed coat. Concentrated sulfuric acid works fine for this. Scarification makes a big difference to germination rate, and is critical for getting synchronous germination. *M. truncatula* also supposedly requires stratification (cold treatment) for uniform germination and growth; however the seed we have worked with in the Stockinger lab (Jemalong) has germinated fine without this stratification treatment.

A. Scarification:

1. Place seeds into a disposable 15ml conical tube.

Take CAUTION when doing the following. Perform this operation in the fume hood and wear protective clothing, gloves, lab coat and safety glasses.

2. Add ~5ml of concentrated sulfuric acid, enough to submerge the seeds. Note however that because the seeds are less dense than the sulfuric acid they will float.

3. Occasionally invert and finger vortex the seeds over an approximate 10min period. The seeds will develop brown specs on them as the acid dissolves the outer hard seed coat. This is fine but don't leave the seed in the acid for too long or it will kill the embryo.

4. Remove the acid using a Pasteur pipette or via aspiration and place it into a second beaker containing a substantial volume of water; **Remember: do like you oughta, add acid to wauta (water)**. You can try decanting the sulfuric acid into this beaker but because the seeds are less dense than the sulfuric acid they tend to float off.

5. Add ~10-15ml of sterile distilled water to the seeds and mix gently.

5. Decant the water and add fresh sterile distilled water.

6. Repeat washing step 3-5 times.

7. Place sterile filter paper in petri plates and add enough sterile distilled water to make the paper damp. Spread the seeds on sterile filter paper and near a source of light.

8. The seed should germinate in 3-5 days. Check occasionally to ensure that they don't dry out. Add some more sterile water if necessary. When they are large enough to handle, transplant them to soil.

B. <u>Stratification</u>:

1. Place the petri dish from step 7 above at 4^{o}C. Leave them at 4^{o}C for about 14 days. Check occasionally to ensure that they don't dry out. Add some more sterile water if necessary.

2. Remove seeds from the cold and leave at room temperature in the light for 2 days before planting.

<u>Note</u>: If sterile plant material is being generated for plant tissue culture and if the acid treatment procedure does not fully surface sterilize the seed, an additional bleach treatment step is essential. Add 30% bleach plus one drop of tween-20 and incubate with occasional inversion and finger vortexing over an approximate 10min period followed by a minimum of 3 sterile ddH_2O rinses.

The original procedure was obtained from Carol Andersson at CSIRO, who received it from Maria Harrison's lab at the Noble Foundation in Oklahoma.