Rooting Media Preparation for *Artemisia tridentata* ssp. *tridentata*

The purpose of this protocol is to prepare media to induce rooting in *A. tridentata* shoot tip cuttings. The volumes included are to make 500 mL of medium, ~50 mL is poured into 12cm x 12 cm culture plates. Medium contains ½ MS + vitamins, 1% sucrose, 1 mL L-1 PPM, 3% phytagel, and 0.5 or 1.0 mg L-1 1-Naphthaleneacetic acid (NAA) or Indole-3-butyric acid (IBA) with a pH of 5.7.

**Equipment:** **Reagents:**

12cm x 12cm square culture plates Phytagel

pH meter Murashige & Skoog w/ Gamborg vitamins

Autoclave indicator tape Sucrose

1L autoclavable bottle or flask Potassium Hydroxide 0.1M

Large graduated cylinder Preservative for Plant tissue culture Media (PPM)

Stir plate w/ stir bar 1 mg mL-1 NAA stock solution

Dropper 1 mg mL-1 IBA stock solution

Scale

Weigh boats

Spatula

1 mL pipette w/tips

**Step 1:**

* Label 1L bottles or flasks for media preparation

**Step 2:**

* Label 1L bottle or flask for each treatment and add ~400 mL DI water using graduated cylinder. Place on stir plate with stir bar and turn on. No heat is needed.
* Weigh 1.1 g MS + vitamins into weigh boat and add to flask.
* Weigh 5 g sucrose into weigh boat and add to flask.
* Pipette 500 μL PPM to solution in flask.

**Step 3 (pH correction):**

* Once media and sucrose are dissolved bring up to volume with DI water.
* Place **calibrated** pH meter into solution (see separate document on how to calibrate pH meter).
* Add 0.1M KOH dropwise to flask until pH meter reads 5.7
  + NOTE: solution has little buffering capacity so wait for pH meter to stabilize before adding more 0.1M KOH. 5 drops at a time works well at this volume.
  + It is OK for pH to be a bit higher because autoclaving will slightly reduce pH value.
* After proper pH is reached remove pH meter, rinse with DI water and then return pH meter to buffering solution container.
* After pH correcting, add 1.5 g phytagel and swirl bottle/flask.

**Step 4:**

* Place caps on bottles/flasks
  + DO NOT screw caps on tightly. Caps should be loose to allow for release of pressure. Bottles should also never be filled to more than 75% capacity.
* Deliver to media prep room for autoclaving. This step takes an hour if they are able to autoclave them immediately.
* After autoclaving, phytagel may still appear separated from medium. Close lids and swirl carefully to mix.
  + NOTE: Bottles/flasks must not be opened unless under laminar flow hood, especially since this solution contains sucrose (more susceptible to contamination).

**Step 5: Pouring medium into culture plates**

* Medium must be cooled to 45-55°C prior to the addition of plant growth regulators.
  + Bottles can be placed at room temperature, in water bath or cooled in ice buckets to reach desired temperature.
  + If medium becomes too cool and solidifies it cannot be re-heated and must be prepared again.
* When medium reaches optimal temperature, add plant growth regulators to reach desired treatment concentration. Swirl medium thoroughly to mix.
  + Example: For 1.0 mg L-1 IBA and NAA treatments, add 500 μL of 1 mg mL-1 stock solution. For 0.5 mg L-1 IBA and NAA treatments, add 250 μL of 1 mg mL-1 stock solution. \*NOTE: these volumes are for 500 mL of medium.
* Label 12cm x 12cm culture plates prior to pouring medium.
* Pour ~50 mL of medium into each culture plate in the laminar flow hood.
* Cool plates with lids off for ~30 minutes. Place plates back into sleeve and store right side up at room temperature prior to use. Plates should ideally be used within 4 weeks.

**Reagents**

**Phytagel:** CAS 71010-52-1; Sigma; powder

**Murashige & Skoog w/ Gamborg vitamins:** M404; Phytotechnology laboratories; phytolab.com; powder

**Preservative for Plant tissue culture Media:** aka PPM; plant cell technology; plantcelltechnology.com; liquid

**Sucrose:** Brand C&H sugar; granules

**Potassium Hydroxide 0.1M:** liquid

**1-Naphthaleneacetic acid:** CAS 86-87-3; Sigma; powder

**Indole-3-butyric acid:** CAS 133-32-4: Sigma; power