

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:

12cm x 12cm square culture plates
pH meter
Autoclave indicator tape
1L autoclavable bottle or flask
Large graduated cylinder
Stir plate w/ stir bar
Dropper
Scale
Weigh boats
Spatula
1 mL pipette w/tips

Reagents:

Phytagel
Murashige & Skoog w/ Gamborg vitamins
Sucrose
Potassium Hydroxide 0.1M
Preservative for Plant tissue culture Media (PPM)
1 mg mL⁻¹ NAA stock solution
1 mg mL⁻¹ IBA stock solution

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, phytigel 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

Phytigel: 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