**Switchgrass Node Rooting Medium with 1mg/l iba and optional 2.5mg/l benomYl**

MS Basal Medium with Gamborg's Vitamins (Sigma)

D-(+) Maltose monohydrate powder, suitable for plant culture (Sigma)

dd H2O

Phytoblend plant agar (Caisson)

Indole-3-Butyric Acid (IBA) Sigma

Benomyl (Sigma) 5mg/ml aliquots (dissolved in DMSO)

1 liter beaker

1 liter graduated cylinder

1 liter bottles with screw caps

100 ml graduated cylinder

microfuge tube (~1.5ml)

pH meter and calibration standards

1 M KOH, 0.1M KOH

1 N NaOH

Stirring platform and stir bars

Heated 57 deg.C water bath

Autoclave/autoclave tape

PhytaTray II containers

100ml or 250 ml Filter Flask, Sigma

Aluminum Foil

**IBA Solution -** prepare stock solution of 100mg/L: dissolve 10 mg IBA powder in a few drops of 1 N NaOH in a microfuge tube. Add ~0.75 ml dd H2O to tube and then pipette that solution into a 100 ml graduated cylinder. Continue adding dd H2O to microfuge tube several times and pour liquid into cylinder until certain that all IBA has been rinsed from microfuge tube. Bring volume to 100ml with dd H2O. Filter sterilize using filter flask assembly. Wrap bottle with aluminum foil and store at 4 deg. C.

**(Benomyl:** Use 5 mg/ml Benomyl (in DMSO) aliquots.) See \*Note at bottom.

**To prepare 1 liter medium:** weigh MS medium amount needed for 1 liter and place in 1 liter beaker containing ~800ml ddH2O and stir bar. Add 30 g D+ maltose, and dissolve. Adjust pH to 5.5 with 1M (and 0.1M) KOH. Add medium to 1 liter cylinder and bring volume to 1000ml.

Add 4.0 Phytoblend agar to each of two 1000 ml bottles. Add 500ml MS-M medium to each bottle. Autoclave 45 minutes on liquid cycle. Cool to ~57 deg. C in heated water bath.

In hood, add 5.0 ml IBA to each bottle containing 500ml of medium. This will equal 1.0mg/L IBA. If using Benomyl, thaw Benomyl (5mg/ml) aliquots and add 250ul to medium (final concentration: 2.5mg/L). Swirl to mix, and pour/divide into PhytaTray II containers. 1 liter medium will be sufficient for 7-8 PhytaTrays. Once solid, store at 4 deg. C.

\*Note on Benomyl: If using nodes from greenhouse or field plants, Benomyl should be used in all media to control for the endophytic fungus *Sarocladium strictum*. If nodes are from plants grown in growth chambers and previously determined to be Sarocladium-free, I suggest as a safeguard, to add Benomyl at 5mg/L initially **only** in petri plates containing Propagation Medium. In the future, it may not be necessary to use Benomyl.