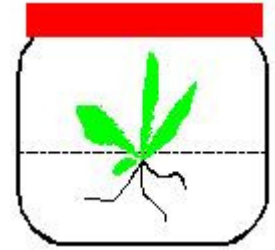


Kitchen Culture Kits Inc.
Protocols for the Hobbyist
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“Bringing plant tissue culture to the classroom and home since 1998”

NOTE: All protocols are intended to be used following the methods described in the KCK Manual: “Plant Tissue Culture for the Classroom and Home” or the online KCK workshop handout.

American Ginseng Tissue Culture

Adapted from Andrew Wang. 1990. Callus induction and Plant Regeneration of American Ginseng. HortScience 25 (5): 571-572.

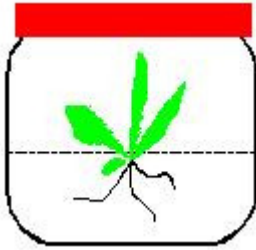
MEDIA

Callus Initiation Medium:

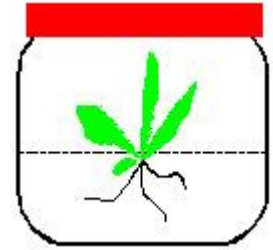
In a 1 liter container, combine the following:

- _____ Distilled water – 2 cups or about 500 ml
- _____ MS Basal Medium with vitamins (1 teaspoon from 1 liter packet)
- _____ PPM - 1 ml
- _____ Sucrose (table sugar) – 2 tablespoons
- _____ 24D – 2 ml (using solution of 1 mg/ml)
- _____ Kinetin – 1 ml (using solution of 1 mg/ml)
- _____ Thiamine HCl – additional 9 mg
- _____ Casein hydrolysate – 100 mg
- _____ Optional: Replace 24D and kinetin with 1.5 mg Dicamba.

Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add one level “pinch” spoon of agar per jar. Sterilize via microwave or pressure cooker as described in the KCK manual.



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Maintenance Medium:

In a 1 liter container, combine the following:

- _____ Distilled water – 2 cups or about 500 ml
- _____ MS Basal Medium with vitamins (1 teaspoon from 1 liter packet)
- _____ PPM - 1 ml
- _____ Sucrose (table sugar) – 2 tablespoons
- _____ 24D – 2 ml (using solution of 1 mg/ml)
- _____ Thiamine HCl – additional 9 mg
- _____ Casein hydrolysate – 100 mg

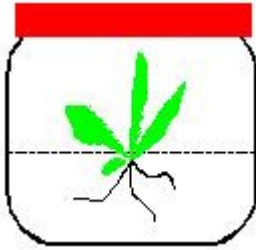
Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add one level “smidgen” spoon of Gellan Gum per jar. Sterilize via microwave or pressure cooker as described in the KCK manual.

Regeneration Medium:

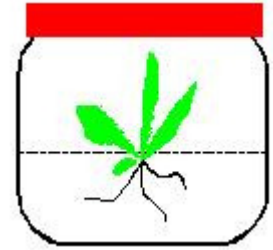
In a 1 liter jar, combine the following:

- _____ Distilled water - 2 cups or about 500 ml
- _____ MS Basal Medium with vitamins (1 liter packet)
- _____ PPM - 1 ml
- _____ Sucrose (table sugar) - 1 1/2 tablespoons
- _____ IBA – 0.5 ml (using solution of 1 mg/ml)
- _____ NAA – 0.1 ml (using solution of 1 mg/ml – can use 6 drops)

Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add one level “pinch” spoon of agar per jar. Sterilize via microwave or pressure cooker as described in the KCK manual.



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ISOLATION AND CULTURE OF EXPLANT

1. Four year old field grown ginseng root was washed in 70% alcohol for about a minute and then blotted with towels. **NOTE: CMS recommends first washing the root with soapy water using a tooth brush or similar to scrub it. You could also keep the root under running water for an hour or so after scrubbing.**
2. The root was then sterilized in 2.5% sodium hypochlorite plus a few drops of dish detergent for 20 minutes. Commercial bleach is 6-8% sodium hypochlorite so a 2.5% dilution is roughly a 1:1 dilution. **NOTE: The original paper reported doing this step under vacuum with occasional agitation.**

MOVE BLEACH SOLUTION/EXPLANTS TO CLEAN BOX

3. In the cleanbox, the root is sliced into 2 mm sections or as thin as possible.
4. The pith tissue of each section is removed with a cork borer 1 cm in diameter and transferred to Callus Induction Medium AND incubated in the dark at 27 C or room temperature. Dark greenish compact calli should develop with 4 weeks.
5. After 4 weeks, the calli are transferred to Maintenance Medium. Friable yellowish callus forms with 2-3 weeks. Incubate in darkness.
6. Dicamba was substituted for 24D to enhance callus growth and induce embryogenesis. Incubate in darkness. **NOTE: Korean ginseng grows better on 24D.**
7. Embryogenic callus was transferred to MS medium with B5 Vitamins, 0.4 mg NAA and 1.0 24D with 30 sucrose and 7 g agar. Incubate in 16 hr light.
8. Somatic embryos matured in 3 weeks and were then transferred to MS medium with 1-2 mg GA3. However, regeneration medium on page 1 does not include GA3.
9. **NOTE: KCK has not tested this protocol.**