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 workshop handout.

Culture of Sequoia sempervirens Shoot Tips and Stem Segments

Adapted from "Plants from Test Tubes". 2013. Kyte et al. page 217.

MEDIA

Initiation Medium:
In a 1 liter container, 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) – 2 tablespoons
_____ Sodium Phosphate Monobasic – 160 mg (use 10 ml of 16 mg/ml solution)
_____ Adenine Sulfate – 80 mg (use 10 ml of 8 mg/ml solution)
_____ Kinetin – 2 mg (use 2 ml of 1 mg/ml solution)
_____ IAA – 0.5 mg (use 0.5 ml of 1 mg/ml solution)

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 1 level "smidgen" spoon of Gellan Gum to each jar. Sterilize via microwave or pressure cooker as described in the KCK manual.

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Rooting Medium **(DO NOT MAKE UNTIL EXPLANTS ARE READY FOR NEW MEDIUM):**
In a 1 liter MICROWAVABLE BEAKER OR MEASURING CUP, combine the following:

_____ Distilled water - 2 cups or about 500 ml
_____ MS Basal Medium with vitamins (1 teaspoon from the 1 liter packet)
_____ PPM - 2 ml
_____ Sucrose (table sugar) – 1 tablespoon + 1 teaspoon
_____ IAA – 2 mg (use 2 ml of 1 mg/ml solution)
_____ IBA – 3 mg (use 3 ml of 1 mg/ml solution)

1. Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8.

2. Use a container that is microwave-proof. A one liter measuring cup works well. See photo. These are easier to handle if only 500 ml is mixed at one time.

3. Pour 500 ml of the liquid **Rooting Medium** into the 1000 ml measuring cup.

4. ADD 11 level "smidgen" spoons of Gellan Gum to the 500 ml media. Mix well.

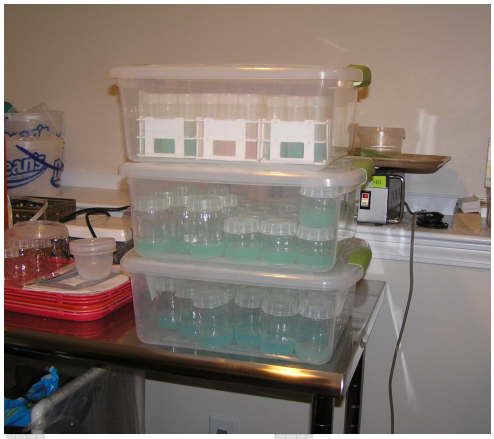
5. Microwave until the Gellan Gum is melted. Look for translucent specs on the side of the container. These are not melted Gellan Gum specs. Microwave for 1 minute more.

6. Now you can add the activated charcoal. THIS medium requires about **600 mg activated charcoal** per liter. Charcoal is VERY MESSY SO HANDLE WITH CAUTION. WEAR GLOVES AND DUST MASKS. PREPARE IN AN AREA AWAY FROM FOOD, AIR CURRENTS, AND PEOPLE, AS THE CHARCOAL WILL CLING TO EVERYTHING. To accomplish this, add the charcoal (1 level dash spoons plus 1 level "pinch" spoon) to the 500 ml of pH-adjusted liquid media. Mix well.

7. Dispense all media using 2 tablespoons per jar, or pour into jars.

8. Process in microwave or pressure cooker. Be sure to swirl media after removing from the pressure cooker or microwave.

9. Cool. Store all media at room temperature in plastic bags or in plastic storage containers.



ISOLATION AND CULTURE OF EXPLANT

1. Harvest 1-3 cm long shoot tips from sprouts or suckers.

2. Wash shoot tips in water with a few drops of dish detergent. Stirring in this solution for 30 minutes might be helpful (cms).

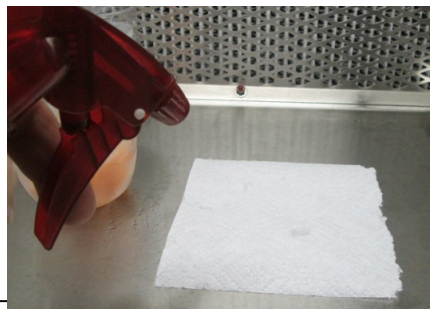
3. Transfer to 70% alcohol (ethanol or isopropanol) and soak for 1-5 minutes. Stir to insure contact of alcohol with the plant tissue.

4. Transfer to a 1/10 solution of commercial bleach (50 ml bleach + 450 ml water + few drops dish detergent.) and soak/stir for 10-20 minutes. Succulent tissues may not tolerate 20 minutes – watch for whitening of tissue (cms).

MOVE BLEACH SOLUTION / EXPLANTS TO THE CLEANBOX.

5. IN THE CLEANBOX: Rinse/soak in sterile water.

6. Spray a piece of paper toweling with 70% ethanol or isopropanol.



7. Aseptically remove one explant at a time and trim exposed ends. Remove any damaged white tissues. Culture on the initiation medium at room temperature and 16 hours light.

8. Shoots will probably develop from existing meristems. These can be excised and placed on the Rooting Medium.

9. Rooted shoots can be planted in perlite/vermiculite (4/1) soil mix.

See original publication for more details. ***Note: KCK has not tested this protocol.***

This article has not been reviewed yet – it should answer many questions:

Sul, IW and SS Korban. 2005.1994. Effect of different cytokinins on axillary shoot proliferation and elongation of several genotypes of Sequoia sempervirens. In Vitro Plant: 30(3): 131-135.