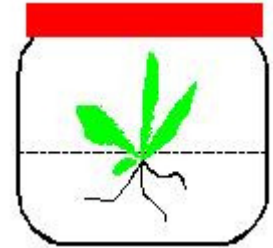


Kitchen Culture Kits Inc.
Protocols for the Hobbyist
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“Bringing plant tissue culture to the classroom and home since 1998”

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.

***Wasabi japonica* “Matsumura”**

Adapted from: C.D. Hung, K. Johnson and F. Torpy. 2006. Liquid culture for efficient micropropagation of *Wasabi japonica* Matsumura. *In Vitro Cell. Dev. Biol-Plant*: 42:548-552.

MEDIA

Initiation Medium:

In a 1 liter container, combine the following:

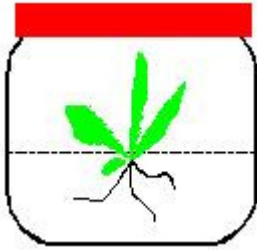
- _____ Distilled water – 2 cups or about 500 ml
- _____ ½ MS Basal Medium with vitamins (1 teaspoon from 1 liter packet)
- _____ PPM - 1 ml
- _____ Sucrose (table sugar) – 2 tablespoons
- _____ BAP – 0.25 ml (using solution of 1 mg/ml)
- _____ GA3 – 0.75 ml (using solution of 1 mg/ml)
- _____ Streptomycin – 50 mg (*heat labile – must be added after heat processing;*
This can be purchased as a sterile solution from PhytoTech Labs)

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.

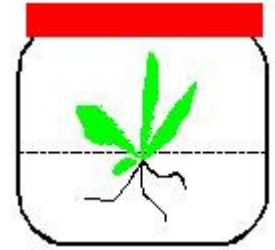
Multiplication Medium:

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
- _____ BAP – 1.1 ml (using solution of 1 mg/ml – 1 ml plus 6 drops)



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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 Gelcarin or carrageenan per jar. Sterilize via microwave or pressure cooker as described in the KCK manual.

Stage III Medium – Rooting:

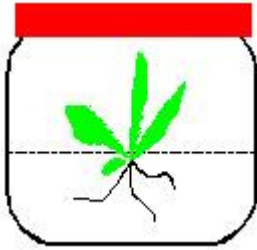
In a 1 liter jar, combine the following:

- _____ Distilled water - 2 cups or about 500 ml
- _____ ½ MS Basal Medium with vitamins (1 liter packet)
- _____ PPM - 1 ml
- _____ Sucrose (table sugar) – 1 1/2 tablespoons
- _____ IBA – 1 or 2 ml (using solution of 1 mg/ml)

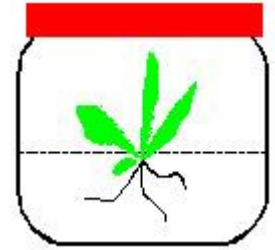
Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add one level "pinch" spoon of agar per jar. Sterilize via microwave or pressure cooker as described in the KCK manual.

ISOLATION AND CULTURE OF EXPLANT

1. Dormant buds of mature rhizomes were collected with a small part of the rhizome attached and washed thoroughly under running water for 40 minutes.
2. These were then soaked in a detergent solution for 5 minutes, rinsed 5 times with distilled water and then soaked in 70% alcohol for 1 minute.
3. The buds were then rinsed with distilled water 2 times and soaked in a 1% solution of sodium hypochlorite for 10 minutes. This is about a 20% dilution of bleach (20 ml bleach + 80 ml water plus few drops detergent). Bleach is usually 6-8% sodiumhypochlorite.



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MOVE BLEACH SOLUTION / EXPLANTS TO CLEAN BOX

4. Transfer explants to sterile water. Rinse 1- 5 times in sterile distilled water.
5. Dormant buds and a small part of the rhizome are cultured on **Initiation Medium**.
6. Single shoots separated from initial cultures were then cultured on **Multiplication Medium**.
7. Elongated shoots were separated and rooted on **Rooting Medium**.
8. Rooted plantlets were later established in soil and acclimated.
9. ***NOTE; The greatest shoot proliferation was achieved using liquid media and a rotary shaker. KCK has not tested this protocol.***