Establishment and Maintenance of Carrot Callus

Carrot [*Daucus carota* L. subsp. *Sativus* (Hoffm.)] is a cool season plant grown for its edible storage taproot. Since the discovery of somatic embryogenesis in carrot culture was made by Steward and Reinert, it has become a model system for investigating many biochemical, physiological, and genetic aspects of plant cell culture. This procedure will outline the method for establishing carrot callus cultures.

Materials Required

1. 10 glass or plastic petri dishes (100 mm in diameter)
2. 2 pairs of forceps and 2 scalpels
3. Sterile #2 cork borer and glass rod that fits it
4. 1000-ml beaker and 250-ml beaker
5. Waterproof marking pen and labels
6. 4 plastic slant racks to hold solid culture tubes
7. Analytical balance
8. Bunsen or ethanol burner
9. 500 ml of 20% Clorox solution supplemented with a few drops of Tween-20
10. 500 ml of sterile distilled water
11. 200 ml of 95% ethanol
12. Carrot Callus Initiation Medium, Product Number C212
13. Carrot Shoot Development Medium, Product Number C222
14. 1 or 2 healthy, undamaged, and regularly shaped carrot roots.

Procedures

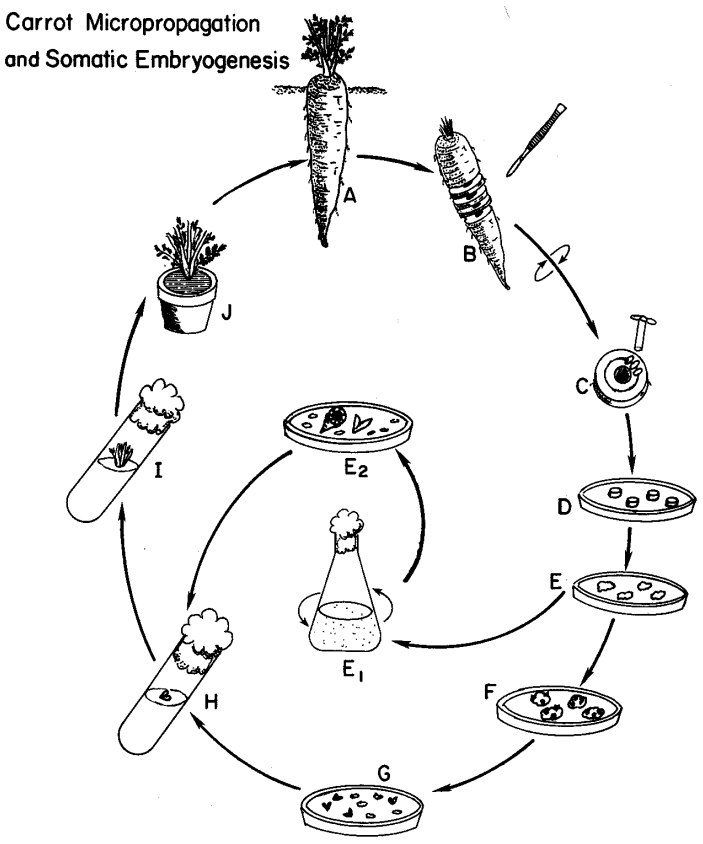
1. Wipe down and turn on laminar flow hood 15 min before doing any work in the hood. Flame-sterilize instruments.
2. Clean carrot root by scrubbing under running tap water to remove any surface soil. Trim the carrot into 100-mm sections and place them in a 1000-ml beaker. Cover with 20% Clorox solution for approximately 20 min and then decant the Clorox solution. Rinse the explant three times in sterile distilled water covering the tissue with each rinse.
3. While carrot sections are being sterilized, prepare and dispense media into culture vessels according to protocols in the media preparation section. Place the culture vessels in racks and label them with respective treatments, medium, and date.
4. Transfer sterilized carrot slices to a sterile petri dish. Using a sterile cork borer, punch out 8-10 cylinders of tissue from the secondary phloem cambial region of the carrot slices. Do not punch out more than two cylinders of tissue at one time, as you may have difficulty in removing them from the cork borer. Using a sterile glass rod, push the cylinders from the cork borer into a sterile petri dish. Using a sharp, sterile scalpel, remove the ends of the tissue cylinder. Next cut the remaining portion of the cylinder into 2- to 3-mm-thick sections. Using a sterile petri plate on the balance, weigh each explant section individually and

inoculate into the culture vessels containing the Carrot Callus Initiation Medium, placing one explant per tube. Repeat this procedure until all culture vessels containing the Carrot Callus Initiation medium have been inoculated. Use a different forcep for each explant and flame the forceps between transfers. Flame the lid of the culture tubes immediately after opening and before closing.

Callus should be removed from the primary explant after 45 days. The calli can be subcultured onto the same medium for further callus growth or it can be subcultured onto the Carrot Shoot Development medium for shoot initiation.

Scheduling

Event	**Timing**
Isolation of explants	Day 0
First subculture	Day 60
Isolation of callus	Day 120



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