Isolation and Culture of Protoplasts from Carrot Cell Suspension Cultures

Early studies indicated that carrot (Daucus carota) cells can be cultured in a liquid medium and their totipotency expressed through somatic embryo differentiation. The successful isolation of protoplasts from carrot cell suspension cultures and the redifferentiation of plantlets from protoplast-derived calli also has been reported (1). Later modifications in the hormone components of the culture medium resulted in induction and formation of somatic embryos without the formation of callus as an intermediate stage. Because of the high morphogenetic potential of carrot cell suspension cultures, carrot cell lines offer an excellent experimental system for the analysis of plant cell growth and differentiation and protoplast-mediated genetic manipulation of plants.

The efficiency of protoplasts will be highly dependent on the type and condition of the cell line. The use of newly established cell lines, preferably less than a year old, is suggested if plant regeneration is a basic requirement in the research. Morphogenetic potential is reported to be more stable in wild carrot cultures than in domesticated carrot cultures. Protoplasts isolated from actively dividing cell cultures generally show a higher frequency of protoplast division and growth than do protoplasts from older cultures in the stationary phase.

Materials Required
1. Culture tubes (20 × 150 mm) for callus cultures
2. 125-ml Erlenmeyer flasks for cell suspension cultures
3. Sterile glass or plastic petri plates (15 × 100 mm)
4. Sterile Pasteur pipettes
5. Forceps and scalpels
6. Sterile graduated centrifuge tubes
7. Bunsen or alcohol burner
8. Variable speed shaker
9. Nylon or wire mesh sieves (ca. 40–60 μM)
10. 0.1% mercuric chloride solution
11. Callus induction medium (1 liter)

- B-5 basal salts (macro- and micronutrients): 1
  - Thiamin·HCl: 1.0 mg/liter
  - Myo-inositol: 100.0 mg/liter
  - Sucrose: 30.0 g/liter
  - 2,4-D: 1.0 mg/liter
  - BA: 0.1 mg/liter
  - Agar: 8.0 g/liter

12. Cell suspension medium (1 liter)

- B-5 basal salts (macro- and micronutrients): 1
  - Thiamin·HCl: 1.0 mg/liter
  - Myo-inositol: 100.0 mg/liter
  - Nicotinic acid: 0.5 mg/liter
  - Pyridoxine·HCl: 0.5 mg/liter
  - Sucrose: 30.0 g/liter
  - 2,4-D: 1.0 mg/liter
  - BA: 0.1 mg/liter

13. Protoplast enzyme/isolation medium containing the following components with final pH of 5.6:

- 2% Onozuka R-10 Cellulase or 2% cellulysin
- 1% Pectinase
- 0.5% Driselase
- 0.5% Rhozyme
- 0.35 M Sorbitol
- 0.35 M Mannitol
- 3 mM 2-(N-morpholino) ethane-sulfonic acid (MES)
- 6 mM CaCl₂·2H₂O
- 0.7 mM NaH₂PO₄·H₂O

14. Protoplasts culture medium

- B-5 basal salts (macro- and micronutrients): 1
  - Thiamin·HCl: 1.0 mg/liter
  - Myo-inositol: 100.0 mg/liter
  - Nicotinic acid: 0.5 mg/liter
  - Pyridoxine·HCl: 0.5 mg/liter
  - Glucose: 68.4 g/liter
  - 2,4-D: 0.5 mg/liter
  - Zeatin: 0.11 mg/liter

1 Prepared as described in Chapter 2 or prepackaged salts.