

optimized. Using the stated conditions, the approximate time from isolation of embryos to having transgenic seed from Taipei 309 varies from 6 to 9 mo.

2. Materials

2.1. Embryo Isolation

1. Single-edge razor blades.
2. Bleach (50%), prepared by diluting commercial bleach (5.25% HClO) with an equal volume of sterile water just prior to use. Add two drops of Tween-20 per 100 mL.
3. Sterile jewelers forceps (e.g., cat. no. 4380, Hamilton Bell, Montvale, NJ).
4. Betadine (10% Iodine antiseptic solution).
5. Sterile 100 × 15-mm culture plates.
6. Sterile 100 × 25-mm culture plates.
7. Ethanol (EtOH, 70%) in a spray bottle.
8. LS 2.5 tissue-culture medium (**14**):
 - a. To 990 mL ultrapure H₂O, add the following, with stirring: 1 package Murashige & Skoog Salt Mixture (**[15]**, cat. no. 11117-066, Gibco-BRL, Gaithersburg, MD), 30 g sucrose, 100 mg myo-inositol, 0.4 mg thiamine-HCl (0.4 mL of a 1-mg/mL solution in H₂O), 2.5 mL of a 1-mg/mL 2,4-D solution (2,4-dichlorophenoxyacetic acid, cat. no. D 8407, Sigma, St. Louis, MO; dissolved in 0.02M NaOH or 95% EtOH). Adjust pH to 5.8 with 0.5M KOH.
 - b. Add 3.85 g agarose (Sigma Type I, cat. no. A6013) per 1-L media bottle, and add medium.
 - c. Leave bottle lid loose, cover lid and neck with aluminum foil, and autoclave (121°C, 25 min, liquid cycle). After autoclaving, swirl medium to evenly distribute agarose and cool to 50°C.
 - d. Transfer bottles to a laminar flow hood, spray, and wipe with 70% EtOH.
 - e. If needed, add filter sterilized selective agent (e.g., 4 mg bialaphos/L; *see Subheading 2.3.*)
 - f. Pour approx 20–25 mL per 100 × 15-mm plate used for embryo isolation and bombardment. For selection plates, pour approx 50 mL per 100 × 25-mm plate.
 - g. Label plates and leave overnight in a laminar flow hood (with fan off) to dry.

2.2. Biolistics (Four Plates of Embryos, Four Shots Per Plate)

1. Bio-Rad helium-driven PDS1000/He system.
2. Grade 5 helium gas in a cylinder with at least 1500 psi.
3. 250 mL 70% EtOH, prepared just prior to use.
4. 100 × 25-mm sterile tissue-culture plates.
5. Eight 1100-psi burst disks (Bio-Rad).
6. Eight 1300-psi burst disks (Bio-Rad).
7. Sixteen macrocarrier holders (Bio-Rad).
8. Sixteen macrocarriers (Bio-Rad).
9. Sixteen stop screens (Bio-Rad).