

4. Shooting medium 2: MS30, 1 mg/L BAP, 100 mg/L kanamycin sulfate, 200 mg/L cefotaxime. Add the appropriate volumes of the stock BAP and antibiotic solutions to the molten MS30 when it has cooled to 50–60°C.
5. Rooting medium: MS30 plus 200 mg/L cefotaxime and 100 mg/L kanamycin sulfate. Add the appropriate volumes of the antibiotic solutions to the molten MS30 when it has cooled to 50–60°C.

2.3. Bacterial Culture Medium

1. Luria-Bertani (LB) broth: 10 g/L tryptone (Difco, Detroit, MI), 5 g/L yeast extract (Difco), 5 g/L NaCl. Dissolve the solids, adjust the pH to 7.2 with 1N NaOH, and autoclave (121°C, 20 min). The broth can be stored at room temperature for several weeks.
2. LB agar: Make up LB broth as described above, then aliquot into bottles, add 15 g/L agar (Becton and Dickinson), and autoclave (121°C, 20 min). The agar can be stored at room temperature for several weeks.

2.4. Bacterial Strains

1. *A. tumefaciens* strain LBA4404 (3).
2. *Escherichia coli* helper strain pRK2013 (4).

2.5. Miscellaneous Solutions

1. 70% (v/v) Ethanol.
2. Bleach solution: 10% (v/v) sodium hypochlorite, 0.05% (v/v) Tween-20.
3. Sterile dH₂O.
4. 10 mM MgSO₄.

2.6. Plant Growth Containers

The seeds should be germinated and grown for 2–3 wk in deep 9-cm Falcon® Petri dishes (Becton and Dickinson, Loughborough, UK). The transformed shoots should be grown initially in 60-mL polypots (Northern Media, Loughborough, UK). The seedlings can then be transferred to larger (250–500 mL vol) vessels. Kilner jars are ideal, and plastic Magenta boxes are available from Sigma. In either case, there should be good ventilation to prevent the buildup of ethylene, which may inhibit plant growth (5). Therefore, the lids should not be tightened, or they can be removed completely and replaced with one half of a Petri dish and sealed with gas-permeable Micropore® tape (3M, St. Paul, MN).

2.7. Miscellaneous Materials and Equipment

1. Sterile filter paper.
2. Sterile Petri dishes.
3. Sterile polypot or beaker.
4. Scalpel.