binations in the culture medium can be used to induce multiplication of undifferentiated callus prior to induction of shoot formation (*see* **Note 1**).

Agrobacterium-mediated transformation has been employed successfully with a number of different potato cultivars ***(4–6)***, using leaf ***(4,7)***, stem ***(7–9)***, or tuber disks ***(5,6)*** as explant tissue. Continuous availability of healthy, vigorously growing leaf and stem material by micropropagation of shoot cultures in vitro is a major advantage over the use of field-grown tubers, for which age is a critical factor in their transformability.

This chapter details a transformation protocol for the cultivar Désirée, using in vitro cultured stem explants, that is rapid and efficient. Désirée is amenable to tissue culture and has a higher capacity for transformation and regeneration than many other potato cultivars, which may require alternative or additional conditions (*see* **Notes 1** and **2**).

2. Materials

2.1. Transformation Vector System

1. An avirulent strain of *A. tumefaciens*, for example, LBA4404, containing the disarmed helper plasmid pRAL4404 ***(10)***.
2. A binary vector, such as pBin19 ***(11)***, with the gene(s) to be transferred integrated into the multiple cloning site between the T-DNA border repeats.

2.2. Bacterial Culture Media

1. LB (Luria-Bertani) broth: 10 g/L bacto-tryptone (Difco, Detroit, MI), 10 g/L sodium chloride, 5 g/L bacto-yeast extract (Difco); make up in deionized water and alter to pH 7.0 with 1*N* NaOH.
2. LB agar: composition as for LB broth; solidify with 15 g/L bacto-agar (Difco).

   Sterilize the media by autoclaving (121°C, 15 min) and add the appropriate antibiotics when it has cooled to 45–55°C. The *A. tumefaciens* strain LBA4404 is resistant to rifampicin (use at 100 mg/L medium), and the binary vector pBin19 confers bacterial and plant cell resistance to kanamycin (for bacterial medium, use at 50 mg/L).
3. Incubator and shaker set at 29°C for *Agrobacterium* cultures.

2.3. Plant Material

1. Virus-free in vitro stock of the potato cultivar. To maintain healthy material, subculture every 4–6 wk by transferring the top 1.5 cm of the shoot (containing the apical meristem) onto fresh MS30 medium in either 30 mL sterile plastic tubes, glass culture tubes (Sigma, St. Louis, MO), or Magenta vessels (Sigma).
2. Growth incubator or room, set at 25°C with a 16-h photoperiod, and a photon flux density of approx 50 μmol/m^2/s, from fluorescent lighting.

2.4. Plant Culture Media

1. MS liquid: Murashige and Skoog basal medium (**ref.** ***12***; Sigma) with 30 g/L sucrose; make up in deionized water and adjust to pH 5.9 with 1*N* KOH.