

appeared to be a very crude method when it was first published in 1987 (6), its potential and effectiveness have become increasingly recognized. For wheat transformation, because there is no versatile biological vector available, and wheat protoplast culture is still an empirical, time-consuming, and genotype-dependent procedure, the microprojectile bombardment of intact tissue, particularly scutellum, became a favorable choice. Third, the *bar* gene, which encodes phosphinothricin acetyltransferase (PAT), was used as the selectable marker, and corresponding substrate, L-phosphinothricin (L-PPT), was used as the selective agent. Although this herbicide selection system does not provide a very “tight” selection, it has been shown to be more effective than some antibiotic selection systems tested. The wheat transformation technique described here is a detailed presentation of these three features, supplemented by the author’s relevant research experience over many years.

2. Materials

1. To maintain a steady supply of plant material, wheat plants (*see Note 1*) are grown in a greenhouse or growth chamber at regular intervals, in 6 inch diameter plastic pots filled with potting mixture (Ready Earth from W.R. Grace or Fisons M2 or F3 compost). Greenhouse and/or growth chamber conditions are as follows: the first 40 d at 15°/12°C d/night temperature and 10-h photoperiod (300 $\mu\text{m}^2/\text{s}$), followed by maintenance at 21°/18°C d/night temperature and 16-h photoperiod (300 $\mu\text{m}^2/\text{s}$). Plants are watered every second day and fertilized once a week with 0.4 g/L of soluble greenhouse fertilizer (*see Note 2*). Plant spikes are tagged on the first day of anthesis, so that seeds containing immature embryos at correct stage for culture can be collected later.
2. Plasmid DNA, containing a selectable marker (e.g., *bar*), a reporter (e.g., *gusA*), and genes of interest (e.g., a piece of viral genome sequences encoding coat protein [CP] or replicase), is prepared at a concentration of 1.0 $\mu\text{g}/\mu\text{L}$ in sterile dH₂O. Good quality DNA (free from RNA and protein contamination) should be used (*see Note 3*). Store DNA solution at -20°C.
3. The Biolistic PDS-1000/He Particle Delivery System (Bio-Rad), or a biolistic device using gunpowder cartridge is needed (*see Note 4*). The biolistic device should be situated in laminar flow hood, and the sample chamber of the device should be kept sterile by spraying 70% ethanol before and after use. Rupture disk holder, rupture disks, parts for assembling stopping plate, and sample plate should be sterilized in 70% ethanol and kept under aseptic conditions.
4. Tungsten or gold particles, 1.0 μm in diameter, are usually used (*see Note 5*). Particles in 60-mg aliquot are washed in 100% ethanol (HPLC or spectrophotometer grade) in a 1.5-mL microcentrifuge tube, then suspended in 1 mL sterile dH₂O. A 50- μL aliquot (for 4–8 bombardments) of the particle suspension is pipetted into microcentrifuge tubes (1.5 mL), while vortexing the suspension continually. The tungsten particle aliquots should be stored at -20°C to prevent oxidation; gold particle aliquots can be stored at 4°C.