5. DNA coating solutions: Prepare 2.5*M* $CaCl_2$ and 0.1*M* spermidine (free base) solutions in distilled water. Filter-sterilize both solutions. Spermine at the same concentration can be used as a substitute for spermidine.
6. Media for immature embryo culture are as follows: The basic medium is MS basal medium (Imperial) supplemented by 30 g/L sucrose, 100 mg/L inositol (MS0), and 1 or 2 mg/L (MS 1 or MS2) 2,4-dichlorophenoxyacetic acid (2,4-D). For shoot regeneration, MS0 without any phytohormones or with 1 mg/L each of indole acetic acid and zeatin is used *(see* **Note 6**). For root formation, half-strength MS0 medium (1/2 MS) is used. The media are adjusted to pH 5.8 using 1*M* HCL or KOH, solidified with 0.25% gelrite, sterilized using autoclaves at 120°C for 20 min, and are poured into 9-cm Petri dishes or Magenta cultural vessels. For selection, add 5 mL or 10 mL L-PPT stock solution (*see* **Subheading 3.8.**) into MS0 or 1/2 MS media, mix well, and pour them into 9-cm Petri dishes or Magenta culture vessels.
7. β-Glucuronidase (GUS) histochernical assay solution: 0.5 mg/mL 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (x-gluc). Dissolve 25 mg x-gluc in 3 mL dimethy sulfoxide (DMSO) in a 100-mL beaker, add 50 m*M* of pH 7.0 phosphate buffer with 0.01% Triton X-100, to a final volume to 50 mL. Filter-sterilize the solution and store at –20°C.
8. Selection stock solution: 1 mg/mL, L-phosphinothricin (L-PPT) stock solution in dH_2O (*see* **Note 7**). Filter-sterilize the stock solution and store at –20°C.

3. Methods

3.1. Preparing Wheat Immature Embryos

1. Collect immature seeds 12–14 d after anthesis and surface-sterilize the seeds using 10% Domestos for 20 min, followed by thorough rinsing with sterile water.
2. Dissect the seeds to excise immature embryos, using fine needles under a low-power (magnification ×16) stereo microscope placed in a laminar flow hood. Immature embryos at this stage are about 1.0–1.2 mm long and semitransparent (*see* **Note 8**).
3. Place the immature embryos on the MS2 medium, with the axis in contact with medium (the scutellum facing upward), 20–30 embryos as a cluster in the middle of a Petri dish (*see* **Note 9**).
4. Preculture the embryos at 25°C in darkness for 2 d before they are subjected to bombardment.

3.2. DNA Coating and Loading

1. To an aliquot of 50 μL of the particle solution in a 1.5-mL microcentrifuge tube, add, in order, under continuous vortexing, 5 μL of DNA, 50 μL of $CaCl_2$, and 20 μL of spermidine, and continue vortexing for 5 min.
2. Spin the microcarriers (the DNA-coated particles) in a microcentrifuge for 20 s; remove and discard as much supernatant as possible.
3. Wash the microcarriers with 250 μL of 100% ethanol (HPLC or spectrophotometer grade) by vortexing briefly, and centrifuge for 20 s; remove and dis-