10. Macrocarrier loader (Bio-Rad).
11. Box of laboratory wipes (Kimwipes) wrapped in aluminum foil and autoclaved.
12. Gold microcarriers (1.0 μm) (Bio-Rad).
13. Plasmid DNA encoding a chimeric phosphinothricin acetyl transferase gene ***(16)*** (e.g., we have used either the CaMV 35S [Huntley and Hall, in preparation] or maize ubiquitin 1 [Christensen and Quail, in preparation] promoter, coupled with the nopaline synthase polyadenylation signal, to drive expression in rice).
14. Plasmid DNA encoding the gene of interest to be introduced.
15. 0.1*M* Spermidine. Dissolve the free base in water, filter-sterilize, and store at –20°C. Discard after 1–2 mo.
16. $CaCl_2$ (2.5*M*), filter-sterilize, and store at –20°C, stable for months.
17. 50% Glycerol, filter-sterilize; make up as needed.

2.3. Selection and Regeneration

1. LS 2.5 tissue-culture medium supplemented with 4 mg/L bialaphos.
2. MSD4 tissue-culture medium:
   a. To 985 mL ultrapure H_2O, add the following with stirring: 1 package Murashige and Skoog Salt Mixture (***[15]***, Gibco-BRL, cat. no. 11117-066), 30 g sucrose, 100 mg myo-inositol, 1 mL of MS vitamins + glycine (1000 X stock, *see below*), 0.5 mL BAP (6-benzylaminopurine, Sigma cat. no. B 9395; 1 mg/mL stock in 0.1*M* HCl), 50 μL NAA (naphthalene acetic acid, Sigma cat. no. N 0640; 1 mg/mL stock in EtOH). Adjust pH to 5.80 with 0.5*M* KOH. Add 3.85 g agarose (Sigma Type I, cat. no. A6013) per 1-L media bottle, and add medium. Cover bottle lid and neck with foil, leave lid loose, and autoclave (121°C, 25 min). After autoclaving, swirl medium to evenly distribute agarose and cool to 50°C. Transfer bottles to a laminar flow hood, spray, and wipe with 70% EtOH. Pour approx 50 mL per 100 × 25-mm plate. Leave plates overnight in laminar flow hood (with fan turned off) to dry.
   b. MS vitamins + glycine (1000X stock; store at –20°C, stable for months): To 50 mL ultrapure H_2O, add the following, with stirring: 25 mg nicotinic acid, 25 mg pyridoxine HCl, 5 mg thiamine-HCl, 100 mg glycine.
3. MS0 medium: Prepare as for MSD4, except omit BAP and NAA. After adding the agarose and medium to the media bottle, fully dissolve the agarose by heating (Phytagel, Sigma cat. no. P 8169 may be substituted for agarose). Aliquot 60–65 mL per Magenta box (Sigma cat. no. V8505) and put the box lids on. Autoclave (121°C, 25 min) and transfer Magenta boxes directly to a laminar flow hood to cool. Stacking boxes helps eliminate condensation on the lids.

2.4. Plant Growth

1. Shredded pasteurized peat moss.
2. Coarse vermiculite.
3. 1-gal Plastic pots.
4. 12 × 2-in. Round trays.
5. 1 × 2-mm Mesh nylon screen.