

2. Insert a 7 × 7-in. nylon mesh into the bottom of a 1-gal pot, and fill with potting soil to within 4 mm of the rim.
3. Make a small depression in the center of the potting soil.
4. Carefully remove a transgenic plant from a Magenta box.
5. Remove all tissue-culture medium from the roots by rinsing them under running water.
6. Remove any callus tissue or dead material from the roots and the base of the plant.
7. Place the plant in the depression with the roots spread out, cover the roots with soil, and gently, but firmly, press down, so that roots and soil make good contact.
8. Label the pot and loosely cover the plant and top portion of the pot with a 1-gal plastic bag.
9. Immediately place the pot into a tray of water.
10. Place plant in a cool, low-light growth chamber (25°C day, 23°C night; 20,000 lux, 14 h light:10 h dark cycle). Replenish water in tray as needed to keep it full.
11. After 2 wk, cut the corners off the plastic bag (leaving approx 2-in. openings) to lower the humidity inside.
12. 3 wk after potting, remove the bag and begin fertilizer regimen:
  - a. For the first 30 d, the plants should be top-fed with 0.1X Mikkelson's solution (*see* **Table 1**). Alternatively, Peters Professional Hydro-Sol fertilizer, as modified (**18**), can be used.
  - b. Thereafter, bottom feed by topping the tray off, as needed, with full-strength (1X) Mikkelson's solution or fertilizer mix.
13. After at least another week (4 wk total) under low-light conditions, adjust the growth chamber to high light (30,000 lux, 16 h light:8 h dark cycle) for at least a week, to complete acclimation. During this period, the very fine leaves produced in the Magenta box die, and broader, normal leaves develop. Confirmation of bialaphos resistance can be tested after the plants have adapted to the soil (*see* **Note 10**).
14. Preferably, transfer plants to a greenhouse. The plants should flower in ~8 wk after potting to soil, and the seeds will be mature ~30 d after pollination (*see* **Note 11**).

#### 4. Notes

1. Since seed development is more advanced at the top of the panicles than the bottom, only ~30–40% of the seeds from each panicle will be at the correct stage. Therefore, for each 100 embryos needed, collect three panicles.
2. The final precipitation of the DNA before coating the particles must be done under aseptic conditions. The DNA volume added to coat the particles may be critical (**4**).

The ratio of selectable marker DNA to gene of interest DNA and the total amount of DNA to coat onto the particles are parameters currently being optimized. There are tradeoffs to be considered when modifying either parameter. We expect that a higher proportion of gene of interest DNA will result in an