(singles cells or cell clusters) will appear on the scutellum tissue of the embryos after staining. Record the number and distribution of the blue spots (per embryo or per bombardment) to obtain transient gene expression frequency data. Such data provide information on the effectiveness of the DNA delivery (*see* **Note 15b**).
3. Continue to culture the rest of the bombarded embryos at 25°C in darkness for another 2 wk.

3.5. Selection and Regeneration of Transformed Shoots

1. After 2–3 wk culture, callus will form from the scutellum tissue of the immature embryos. Transfer these embryo–callus cultures to MS1 medium containing 5 mg/L L-PPT in 9-cm Petri dishes and place the cultures at 25°C under fluorescent light (3000 lux for 16 h). Presence of L-PPT in the MS1 medium allows transformed cells and cell clusters to proliferate, while suppressing the growth of the untransformed cells (*see* **Note 16**).
2. Allow these embryo–callus cultures to grow for another 2–3 wk, somatic embryo structures and green spots will appear on some of the calli. Transfer these calli to MS0 medium containing 5 mg/L L-PPT in 9-cm Petri dishes for shoot development; leave the rest of the calli to continue to grow until they form morphogenic structures.

3.6. Selection and Regeneration of Plantlets

1. After a further 2–4 wk, shoots will form on the L-PPT containing MS0 medium. Shoots normally grow in clumps. When their leaves reach 1–2 cm, dissect the shoot clumps into individual shoots, transfer them into 1/2 MS medium containing 10 mg/L L-PPT in Magenta culture vessels (4–5 shoots into a box), and culture them under the same growth condition.
2. Truly transformed shoots will produce healthy roots at the base of the shoots, to form the transgenic plantlets (T_0 plantlets). Allow the T_0 plantlets to grow until their leaves reach to the lid of the Magenta culture vessel. A small proportion of untransformed shoots, which escaped from the first two rounds of selection, will have bleached leaves and will not form healthy root system (*see* **Note 17**).
3. For winter varieties, the T_0 plantlets can be vernalized at 4°C for 4–8 wk at this stage.

3.7. Growth of T_0 Plants Into Maturity

1. Gently pull the plantlets out of the Magenta culture vessel. With great care, wash away with tap water the Gelrite or agarose surrounding the roots, so that the root system is not damaged.
2. Transfer the plantlets into pots containing Fisons F3 (Fisons) compost. It is useful to provide these plantlets with a low-humidity condition (about 30–50%) in a growth chamber for 1–2 wk, then transfer them into a contained greenhouse.
3. Maintain these plants in the greenhouse condition as described above (*see* **Subheading 2., item 1**). Cover all of the spikes of the T_0 transgenic plants with crossing bags during the flowering stage (*see* **Note 18**).