4. For the maturation of the seeds of the $T_0$ plants ($T_1$ seeds), cease to water, and feed the plants 30 d after the pollination.
5. Harvest and label the $T_1$ seeds from each individual spike.
6. Autoclave the rest of naturally dried plant parts (straw, old leaves, and roots) and compost for disposal.

### 3.8. Phenotypic, Genetic, and Molecular Analyses of Transgenic Plants and Their Progenies

1. For phenotypic and genetic analyses, germinate the $T_1$ seeds (and some untransformed seeds as negative control) in 9-cm Petri dishes containing L-PPT solution (10 mg/L, soaked in filter paper). The seeds that have the functional *bar* gene will germinate and produce normal seedlings, but the seeds that do not have the *bar* gene will produce stunted and bleached leaves without a healthy root system.
2. Collect information about the germination frequency based on seeds from individual spikes. If a single *Locus* integration of the *bar* gene had occurred in the $T_0$ plants, a typical Mendelian segregation of 3:1 (resistant:sensitive) would be observed.
3. An alternative test is to germinate the $T_1$ seeds in soil and grow the seedlings for 3–5 wk, then spray the seedlings with 2% Basta. The sensitive plants will show bleached leaves a few days after the spraying and subsequently be killed, but the resistant plants will grow normally. The ratio of resistant to sensitive plants would be 3:1, if transgenes are integrated at a single *Locus* (*see* **Note 19**).
4. Choose L-PPT or Basta resistance plants, collect plant parts at different stages of development, e.g., leaf, root, ovary, anther, and microspore, for GUS assays (*see* **Subheading 3.4.2.**).
5. Detection of PAT enzyme activity can also be conducted ***(8)*** in the L-PPT-resistant $T_0$ and $T_1$ plants.
6. Southern hybridization and polymerase chain reaction (PCR) analyses to confirm stable integration and desired expression of the transgenes (*see* **Note 20** and Chapters 41–43).
7. Northern hybridization analysis to detect the transcripts of the transgenes (*see* Chapter 44).
8. To detect the product of a particular transgene, which is not readily detectable using enzymatic assays, e.g., viral CP or replicase, the Western hybridization analysis is necessary (*see* Chapter 45).

## 4. Notes

1. The choice of wheat variety for the transfommation test is important. So far, only those varieties that are highly regenerable under tissue culture conditions, are suitable for transformation tests. The varieties that are shown to be amenable to transformation include the following varieties: Bob White, Pavon, RH770019, Florida, and Fielder. If transformation targets are commercial varieties, it is recommended that a regeneration test is carried out in advance.