8. After 2 d of incubation, the tomato cotyledon explants are transferred to plates with selective shoot regeneration medium and incubated in a plant growth chamber under the same conditions (*see* **Note 4**).
9. Every week, the cotyledon pieces are placed on fresh selective shoot regeneration medium (*see* **Note 5**). After about 4 wk, depending on the tomato cultivar, the first calli can be observed.
10. Leaf pieces with calli, which have reached a size of ca. 1–2 mm, are transferred individually to plant containers (7 × 10 cm) with selective shoot regeneration medium. This procedure results in a much better growth of the calli (*see* **Note 6**).
11. After about 2 wk, shoots start to grow from the calli. Shoots of ca. 1 cm in size are cut off and placed on selective root regeneration medium in sterile plant containers (7 × 10 cm).
12. Within 1–2 wk, roots start to develop. Rooted shoots are now transferred to soil and further cultivated in the greenhouse (*see* **Note 7**).

4. Notes

1. Stock solutions are all filter-sterilized using a 0.45-μm Millipore filter and frozen in aliquots at –20°C.
2. Acetosyringone and an acidic pH are important for the induction of the *vir* genes of *Agrobacterium.*
3. Since overgrowth of the tomato leaf pieces by *Agrobacteria* is a constant problem with tomato transformation, it is very important to dilute the *Agrobacterium* culture well enough.
4. The transformation efficiency can be enhanced by a factor of about two by using feeder layers of suspension culture cells from tobacco. In our hands, the feeder layers turned out to be a constant source of contaminations. Therefore, we recommend omitting the feeder cells and following the above protocol carefully.
5. A weekly change of the medium is very important to keep the *Agrobacteria* down.
6. It is also important to watch the calli very carefully. If the calli start to produce phenolic compounds, the medium has to be changed immediately. Otherwise, the calli may die within hours.
7. Tomato plants can be easily multiplied by cuttings.

References

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