Potato Transformation

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[Abstract] This is a protocol to produce stable transgenic potato plants (*Solanum tuberosum* cv. Désirée) by *Agrobacterium*-mediated genetic transformation, which is established based on a method described by (Jung *et al*., 2005) with some modifications. *Agrobacterium tumefaciens* strain LBA4404 carrying the desired construct is used to infect internodal explants to produce stable transgenic potato plants. Plantlet screening and molecular analyses are employed to confirm the expression of transgene in generated transgenic potato lines.

Materials and Reagents

1. Potato (*S. tuberosum* cv. Désirée) tissue culture plantlets
2. 70% ethanol
3. Bleach (6% sodium hypochlorite)
4. Tween 20
5. Acetosyringone (Fisher Scientific, catalog number: AC11554)
6. Timentin (*Phyto*Technology Laboratories®, catalog number: T869)
7. Selective antibiotics
8. Mannitol (Fisher Scientific, catalog number: M120)
9. Glycine
10. Nicotinic acid (Sigma-Aldrich, catalog number: N0761)
11. Pyridoxine HCl (Sigma-Aldrich, catalog number: P6280)
12. Thiamine HCl (Sigma-Aldrich, catalog number: T1270)
13. Folic acid (Fisher Scientific, catalog number: BP2519)
14. Biotin (Fisher Scientific, catalog number: BP232)
15. MS salt (Caisson Laboratories, catalog number: MSP01-50LT)
16. Inositol (Fisher Scientific, catalog number: AC122261000)
17. Sucrose
18. 6-benzylamino purine (BAP) (Sigma-Aldrich, catalog number: B3408)
19. 1-naphthalene-acetic acid (NAA) (Sigma-Aldrich, catalog number: N0640)

20. 3-indoleacetic acid (IAA) (Sigma-Aldrich, catalog number: I2886)
21. Trans-zeatin-riboside (Sigma-Aldrich, catalog number: Z0876)
22. YM medium (see Recipes)
23. MSVI vitamins (see Recipes)
24. JHMS vitamins (see Recipes)
25. 3R vitamins (see Recipes)
26. CIM medium (see Recipes)
27. 3C5ZR plates with selective agent (see Recipes)
28. Propagation medium (see Recipes)
29. TPS buffer (see Recipes)

Equipment

1. Sterile magenta boxes (sterilized by autoclaving)
2. Sterile petri dishes (Fisher scientific, catalog number: FB0875712)
3. Sterile forceps and scalpel (sterilized by heat treatment using a Bunsen burner)
4. Sterile glass tubes with caps (sterilized by autoclaving) (tubes, Fisher Scientific, catalog number: 14-961-34; caps, Fisher Scientific, catalog number: 14-957-91D)
5. Sterile inoculating loop (Fisher Scientific, catalog number: 22-363-604)
6. Tissue grinders
7. Glass culture tubes (sterilized by autoclaving)
8. 3M Micropore tape (Fisher Scientific, catalog number: 1530-0 and 1530-1)
9. Agrobacterium glycerol stock
10. 50 ml conical centrifuge tubes
11. Tissue culture biosafety cabinet
12. Incubator shaker (New Brunswick Scientific, model: C24KC)
13. Centrifuge
14. *S. tuberosum* growth chambers (we use several types of growth chambers such as I-66LLVL from Percival)
15. Hot block or water bath
16. PCR thermal cycler (Bio-Rad Laboratories, model: C1000 touch)

Procedure

A. Generation and propagation of potato tissue culture plantlets (performed under sterile conditions)

1. Excise sprouts (at least 2.5 to 4 cm long) from potato tubers or stem segments (containing at least one internode) from plants and surface sterilize these plant pieces:
   a. Wash with 70% ethanol for 1-2 min.
   b. Use a sterile forceps to transfer plant pieces to 10% bleach containing trace amount of Tween 20 (1 µl/ml) and soak for 10-12 min.
   c. Transfer plant pieces to 70% ethanol and rinse for 1-2 min.
   d. Wash with plenty of sterile distilled water to remove any trace of ethanol.
2. Trim the sterilized plant pieces with sterile scalpel to remove damaged cell layers and culture each plant piece on propagation medium in a glass tube at 24 °C under a 16-h/8-h light/dark cycle. Plantlets originated from the sterilized plant pieces are generally established for approximately four to six weeks.
3. Cut approximately 1 cm-long nodal stem segments from plantlets (Figure 1), and push firmly into a magenta box or glass tube containing propagation medium, leaving the top part of the stem exposed. Put six segments per box or one segment per glass tube, and seal the box cover or tube cap with micropore tape. Around twenty four plantlets are suitable for transformation of one construct.
4. Cultivate in a growth incubator at 24 °C under a 16-h/8-h light/dark cycle for four to six weeks.

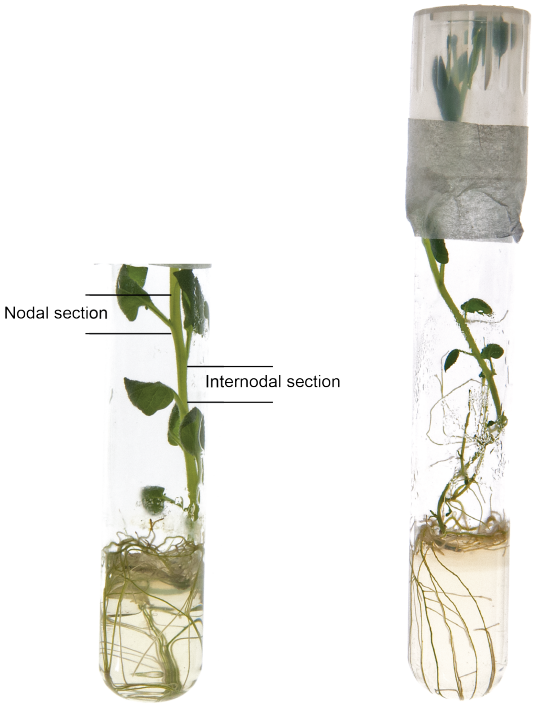

Figure 1. Potato plantlet growing in the propagation medium. Nodal and internodal sections are indicated as shown.

B. *Agrobacterium* preparation

1. Four days prior to infection, streak out *Agrobacterium tumefaciens* LBA4404, carrying the desired construct (transgene), on an YM plate with appropriate antibiotics. Incubate the plate at 28 °C for three days.
2. The day before infection, inoculate 10 ml of YM with *Agrobacterium* colonies and culture at 28 °C on an incubator shaker till OD_{00} reaches 0.7 (usually need overnight culturing).
3. On the day of inoculation, centrifuge the *Agrobacterium* culture at 9,000 *x g* for 15 min at room temperature and re-suspend the pellet in 10 ml CIM liquid medium.
4. Add 50 µl of 0.074 M acetosyringone to the *Agrobacterium* suspension (the final concentration of acetosyringone is 0.37 mM). Use within one hour, as cells will begin to aggregate.

C. Potato transformation (performed under sterile conditions)

1. On the day of infection, cut internodal stem sections (Figure 1) from four to six weeks plantlets into about 1 cm pieces and transfer to CIM plates.
2. Incubate explants with 10 ml *Agrobacterium* inoculum for 20 min, occasionally swirling the plates. We usually use 100 stem pieces per construct.
3. Use sterile forceps to transfer stem pieces from the bacterial culture to new CIM media plates. Label plates, seal with micropore tape, and place them back to the 24 °C incubator in the dark.
4. After three days, transfer stem pieces to 3C5ZR medium plates with appropriate antibiotics and 500 µg/ml timentin. Timentin is used to inhibit the growth of *A. tumefaciens* LBA4404.
5. Place the plates in the growth incubator (24 °C, 16-h/8-h light/dark cycle) and transfer the stem pieces to fresh 3C5ZR medium plates every seven to ten days, until plantlets emerge (Figure 2).
6. When plantlets are at least half a centimeter long, cut them from explants and transfer to culture tubes containing 10 ml of propagation medium with the appropriate antibiotics and timentin at 100 µg/ml. Sterile inoculating loops can be used to gently push the base of the plantlet into the medium. Label and seal the tubes with micropore tape.
7. Grow plants for 3 - 4 weeks until roots are established. Discard any plants that do not grow roots within one month, as they are not transgenic.
8. Cut and re-propagate the transgenic plants as needed.

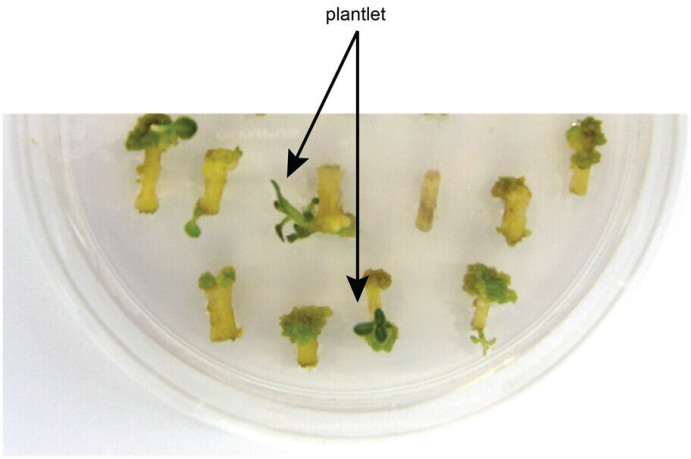

Figure 2. Potato internodal sections cultivated on the 3C5ZR medium. Plantlets (indicated) are emerging from the infected nodal or internodal sections.

D. PCR screening

1. Cut approximately one-half to one square centimeter of leaf tissue to a 1.5 ml centrifuge tube.
2. Add 200 µl TPS to each tube.
3. Grind the tissue in TPS with a tissue grinder to obtain an uniform slurry.
4. Incubate samples at 75 °C for 20 min.
5. Centrifuge at 13,400 *x g* for 10 min.
6. Transfer supernatant to a new tube and add an equal volume of isopropanol to each tube. Mix tubes by inversion.
7. Centrifuge at 13,400 *x g* for 10 min. DNA pellet should be visible at this point.
8. Remove supernatant and wash pellet with 70% ethanol. Centrifuge at 13,400 *x g* for 5 min.
9. Remove supernatant, air-dry pellet and resuspend pellet in 30 µl of sterile distilled water or TE containing RNaseA (100 µg/ml).
10. Use 1 µl of DNA as template for PCR screening of transformants.
11. Typical PCR program:
    First step: 95 °C for 3 min.
    Second step: 94 °C for 30 sec, 58 °C for 30 sec, 72 °C for 1 min x 30 cycles.
    Third step: 72 °C for 5 min.

Recipes

The following media are prepared and stored at room temperature. The poured medium plates containing antibiotics can be stored at 4 °C for up to one month. Vitamins can be prepared, aliquoted and stored at -20 °C.

1. YM Medium (1 L)
   0.4 g yeast extract
   10 g mannitol*
   0.1 g NaCl
   0.2 g $MgSO_4 \cdot 7H_2O$
   0.5 g KH_2PO_4
   10 g Agar (for plates)
   Autoclave
   *Make and autoclave separately, make 10 g/100 ml and use 100 ml/L
2. MSVI vitamins
   2 mg/ml glycine
   0.5 mg/ml nicotinic acid
   0.5 mg/ml pyridoxine HCl
   0.4 mg/ml thiamine HCl
3. JHMS vitamins
   0.4 mg/ml folic acid
   50 µg/ml biotin
4. 3R vitamins
   1 mg/ml thiamine HCl
   0.5 mg/ml nicotinic acid
   0.5 mg/ml pyridoxine HCl
5. CIM medium (1 L)
   4.3 g MS salt
   1 ml MSVI vitamins
   1 ml JHMS vitamins
   0.1 g inositol
   30 g sucrose
   1 mg 6-benzylamino purine (BAP)
   2 mg 1-naphthalene-acetic acid (NAA)
   10 g agar (for plates)
   Adjust pH to 5.6 with KOH and autoclave
6. 3C5ZR medium (1 L)

4.3 g MS salt
1 ml 3R vitamins
0.1 g inositol
30 g sucrose
10 g agar (for plates)
Adjust pH to 5.9 with KOH and autoclave
0.5 mg 3-indoleacetic acid (IAA) *
3 mg trans-zeatin-riboside *
500 mg Timentin*
Selection agent **
* Add after sterilization
** Use appropriate selection agent and concentration for construct

7. Propagation medium (1 L)
   4.3 g MS salt
   0.17 g $NaH_2PO_4 \cdot H_2O$
   0.1 g inositol
   0.4 mg thiamine HCl
   30 g sucrose
   2.5 g gelrite or 10 g agar
   Adjust pH to 6.0 with KOH and autoclave
8. TPS buffer
   100 mM Tris HCl (pH 8.0)
   100 mM EDTA (pH 8.0)
   1 M KCl

Acknowledgments

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