Protocol

TD-P Revision 3.0

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Potato-Agrobacterium Plant Transformation

Adapted from Hui Duan, et al. (2012)

Introduction

Agrobacterium tumefaciens is a gram-negative bacteria and plant pathogen that typically causes tumor-like growths on infected plants. These tumors are prompted by the movement of transfer DNA (T-DNA) from the plasmid to the genome of the host eukaryote (often fungi). This horizontal gene transfer system, called *Agrobacterium tumefaciens*-mediated transformation or *At*MT, has become a useful technique for the insertion of modified DNA into cells to create genetically modified plant lines, including transgenic plants capable of resistance to pathogens normally associated with reduced or unusable harvests. Here, we present a protocol adapted from a study published in 2012 that documented a marked increase in resistance to *Potato virus Y (PVY)* by a potato species following its modification with the eIF4E-1 gene carried via the *Agrobacterium*.

Materials

- *Agrobacterium* cultures
- MS liquid medium
- Sucrose
- Russet Burbank potato plants
- M516 medium
- Gelrite (GoldBio Catalog # G1101)
- CIM medium
- SIM medium
- Agar (GoldBio Catalog # P1001)
- trans-Zeatin-riboside (GoldBio Catalog # Z-100)
- Napthalene acetic acid
- Timentin™ (Ticarcillin/Potassium Clavulanate) (GoldBio Catalog # T-104)
- Kanamycin (GoldBio Catalog # K-120)
- Gibberellic acid GA3 (GoldBio Catalog # G-120)

Callus Induction Media (CIM):

- MS medium
- 3% Sucrose 3
- 2.5 mg/l Zeatin-riboside
- 0.1 mg/l Naphthalene acetic acid
- 6 g/l agar
- 150 µg/ml Timentin™
- 100 µg/ml Kanamycin

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Shoot Induction Media (SIM):

- MS medium
- 3% Sucrose
- 2.5 mg/l Zeatin Riboside
- 0.3 mg/l Gibberellic acid GA3
- 6 g/l Agar
- 150 µg/ml Timentin™
- 100 µg/ml Kanamycin

Method

1. Streak *Agro* cells carrying the elF4E-1 gene from a plate or a stab onto a plate and incubate at 30°C for 36-48 hours to allow to grow.

2. Use a well-isolated colony to inoculate 50 ml of broth in a 500 ml flask and grow cells with vigorous aeration at 30°C until OD_{550} = 0.2.

3. Prepare 10-fold dilutions of overnight-grown *Agro* cultures and grow for 5-6 hours.

4. Centrifuge for 15 minutes at 1,100 g.

5. Discard the supernatant and wash the pellet with MS liquid medium, supplemented with sucrose (3% at pH 5.7).

6. Resuspend in the same medium until OD_{600} reaches ~0.02.

7. Use these resuspended cells to infect 0.4-0.6 mm intermodal segments of Russet Burbank potato plants maintained in magenta boxes containing 40 ml of half-strength M516 medium with 3% sucrose and 2 g/l Gelrite. Swirl the boxes occasionally.

8. Incubate the infected stems for 2 days on co-culture medium (1/10 MS salts, 3% sucrose at pH 5.7) containing 6 g/l agar at 22°C in a Percival growth chamber (16-hours light).

9. Transfer the infected stems to CIM and incubate for 1 month.

10. Transfer the infected stems to SIM until shoots emerge.

11. Once they emerge, transfer the shoots to MS medium with 3% sucrose, 6 g/l of agar and Timentin™ (150 µg/ml).

12. Collect a tissue sample from each plantlet for DNA isolation to confirm T-DNA by PCR.

13. Transfer T-DNA positive plants to soil and subsequently to a growth chamber under 11 hours of light at 25°C.

Associated Products

- Gelrite (GoldBio Catalog # G1101)
- Agar (GoldBio Catalog # P1001)
- trans-Zeatin-riboside (GoldBio Catalog # Z-100)
- Napthalene acetic acid (GoldBio Catalog # N-780)
- Timentin™ (Ticarcillin/Potassium Clavulanate) (GoldBio Catalog # T-104)
- Kanamycin (GoldBio Catalog # K-120)
- Gibberellic acid GA3 (GoldBio Catalog # G-120)

References

Hui, D., Richael, C., and Rommens, C. (2012). Overexpression of the wild potato eIF4E-1 variant Eva1 elicits Potato virus Y resistance in plants silenced for native eIF4E-1. *Transgenic Research* (2012): 1-10.

Jung, C. S., Griffiths, H. M., Jong, D. M., Cheng, S., Bodis, M., and Jong, W. S. (2004). The potato P locus codes for flavonoid 3',5'-hydroxylase. *Theoretical and Applied Genetics, 110*(2), 269-275. Doi:10.1007/s00122-004-1829-z.